07-27-09 09/521-264

SUNSTEIN

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July 24, 2009

Express Mail EV 626471666US

Mail Stop Patent Term Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: Application for Extension of Patent Term Under 35 U.S.C. § 156
Patent No.: U.S. Patent 6,599,498
Our File: 1275/700

Dear Commissioner:

Enclosed please find the following:

1. Original Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J);

2. Certificate of Express Mailing;

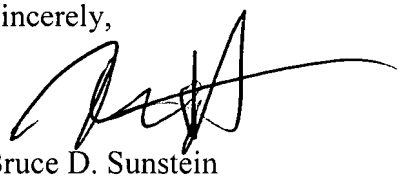
3. Four (4) copies of Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J); and

4. Return Postcard.

Authorization is hereby made to charge the amount of $1,120.00 to Deposit Account No. 19-4972 pursuant to 37 C.F.R. § 1.20(j).

Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

Sincerely,



Bruce D. Sunstein

BDS/

Enclosures

01275/00700 1119252.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	U.S. Patent 6,599,498 ("Heat Stable Colloidal Iron Oxides Coated with Reduced Carbohydrates and Carbohydrate Derivatives")
Issued:	July 29, 2003
Filed:	March 8, 2000
Inventors:	Ernest B.V. GROMAN Kenneth G. Paul Timothy B. Frigo Howard Bengele Jerome M. Lewis
Assignee:	AMAG Pharmaceuticals, Inc.

Mail Stop Patent Term Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL OF APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

1. Applicant submits herewith the following:

Cover Letter for Application for Extension of Patent Term Under 35 U.S.C. § 156;

Original Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J);

Four (4) copies of Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J); and

Return Postcard.

2. The fee set forth in §1.20(j)) ($1,120.00), required by 37 C.F.R. § 1.740(14), is paid as follows:

Authorization is hereby made to charge the amount of $1,120.00 to Deposit Account No. 19-4972.

Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

Date: July 24, 2009

Bruce D. Sunstein
Registration No. 27,234
SUNSTEIN KANN MURPHY & TIMBERS LLP
125 Summer Street
Boston, MA 02110-1618
617-443-9292
Customer No. 002101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: U.S. Patent 6,599,498 ("Heat Stable Colloidal Iron Oxides Coated with Reduced Carbohydrates and Carbohydrate Derivatives")

Issued: July 29, 2003

Filed: March 8, 2000

Inventors: Ernest B.V. GROMAN
Kenneth G. Paul
Timothy B. Frigo
Howard Bengele
Jerome M. Lewis

Assignee: AMAG Pharmaceuticals, Inc.

Mail Stop Patent Term Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Commissioner:

Applicant, AMAG Pharmaceuticals, Inc. (formerly known as Advanced Magnetics, Inc.), acting through its duly authorized attorney, hereby submits this application for extension of patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the PTO (37 C.F.R. § 1.710 - 1.785). For the convenience of the PTO, the information presented in this application is in a format which follows the requirements of 37 C.F.R. § 1.740.

(1) **A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics.**

The approved product, Feraheme™ (ferumoxytol) Injection (hereinafter "the AMAG Product"), known generically as ferumoxytol, is indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease. The AMAG Product is a non-

stoichiometric magnetite (superparamagnetic iron oxide) coated with polyglucose sorbitol carboxymethylether and is formulated with mannitol. The AMAG Product is a black to reddish brown liquid. Each mL of the sterile colloidal solution of the AMAG Product contains 30 mg of elemental iron and 44 mg of mannitol, and has low bleomycin-detectable iron. The formulation is isotonic with an osmolality of 270-330 mOsm/kg. The product contains no preservatives, and has a pH of 6 to 8. The chemical formula of the AMAG Product is $Fe_{5874}O_{8752}$-$C_{11719}H_{18682}O_{9933}Na_{414}$ with an apparent molecular weight of 750 kDa. The active ingredient of the AMAG Product is a non-stoichiometric magnetite (superparamagnetic iron oxide) coated with polyglucose sorbitol carboxymethylether.

(2) **A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.**

The AMAG Product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act Section 505 (21 U.S.C. § 355).

(3) **An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred.**

The AMAG Product received permission for commercial marketing or use under Section 505 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355) on **June 30, 2009** (NDA 22-180). See Attachment A (FDA Approval Letter).

(4) **In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.**

The active ingredient of the AMAG Product is ferumoxytol. The active ingredient has not been previously approved for commercial marketing or use under Section 505 or any other section of the Federal Food, Drug and Cosmetic Act prior to its approval in NDA 22-180 by the FDA. It has not been approved for commercial marketing or use under the Public Health Service Act, or the Virus-Serum-Toxin Act.

(5) **A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted.**

This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the permitted 60 day period pursuant to 37 C.F.R. § 1.720(f), the last day of which is **August 28, 2009**.

(6) **A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration.**

The complete identification of the patent for which extension is being sought is as follows:

Inventors:	Ernest B.V. Groman Kenneth G. Paul Timothy B. Frigo Howard Bengele Jerome M. Lewis
Patent Number:	6,599,498
Issue Date:	July 29, 2003

Practitioner's Docket No. 1275/700 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: U.S. Patent 6,599,498 ("Heat Stable Colloidal Iron Oxides Coated with Reduced Carbohydrates and Carbohydrate Derivatives")

Issued: July 29, 2003

Filed: March 8, 2000

Inventors: Ernest B.V. GROMAN
Kenneth G. Paul
Timothy B. Frigo
Howard Bengele
Jerome M. Lewis

Assignee: AMAG Pharmaceuticals, Inc.

Mail Stop Patent Term Extension
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

EXPRESS MAIL CERTIFICATE

"Express Mail" label number EV626471666
Date of Deposit 07/24/2009

I hereby state that the following *attached* paper or fee

Cover Letter for Application for Extension of Patent Term Under 35 U.S.C. § 156;

Original Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J);

Four (4) copies of Application for Extension of Patent Term Under 35 U.S.C. § 156, with exhibits (A)-(J); and

Return Postcard.

are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10, on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Bruce D. Sunstein

Signature of person mailing paper or fee

01275/00700 1119407.1

Expiration Date: March 8, 2020

(7) **A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings.**

See Attachment B for a complete copy of the patent identified in paragraph (6) hereof.

(8) **A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent.**

See Attachment C for copies of the Certificates of Correction that issued for U.S. Patent 6,599,498 on March 2, 2004 and January 27, 2009, respectively. No re-examination certificate has been issued with respect to U.S. Patent 6,599,498. No disclaimer has been filed with respect to U.S. Patent 6,599,498. Enclosed at Attachment D is a copy of the Maintenance Fee Statement which shows that the first maintenance fee (3 ½ year maintenance fee) has been paid.

(9) **A statement that the patent claims the approved product, or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one such patent claim reads on:**

(i) The approved product, if the listed claims include any claim to the approved product;

(ii) The method of using the approved product, if the listed claims include any claim to the method of using the approved product;
and

(iii) The method of manufacturing the approved product, if the listed claims include any claim to the method of manufacturing the approved product.

The statements provided herein are made solely to comply with the requirements of 37 C.F.R. 1.740(a)(9). As acknowledged by M.P.E.P., 37 C.F.R. 1.740(a)(9) does not require applicant to show whether or how the listed claims would be infringed, and this question cannot be answered without specific knowledge concerning acts performed by third parties.

As such, the following are not assertions or admissions by Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or importation of any product.

U.S. Patent 6,599,498 has claims covering the approved product (claims 12-21 and 25-26) as well as methods of manufacturing the approved product (claims 1-11, and 23-24). The relevant claims[1] of the patent read as follows:

1. A method of providing an iron oxide complex for administration to a mammalian subject, the method consisting of:

producing a carboxyalkylated reduced polysaccharide iron oxide complex; and sterilizing the complex by autoclaving.

2. A method according to claim 1, wherein the reduced polysaccharide is a reduced polymer of glucose.

3. A method according to claim 2, wherein the reduced polymer of glucose is a reduced dextran.

4. A method according to claim 1, wherein the reduced polysaccharide is produced by reacting a polysaccharide with a reagent selected from the group consisting of: a borohydride salt, and hydrogen in the presence of an hydrogenation catalyst.

5. A method according to claim 1, wherein producing the complex includes carboxyalkylating a reduced polysaccharide by carboxymethylation.

6. A method according to claim 5, wherein the reduced polysaccharide is a reduced dextran.

[1] This listing of the claims includes wording that gives effect to language issued in a Certificate of Correction on January 27, 2009.

7. A method according to claim 6, wherein the administration to a mammalian subject is administration to a human.

8. A method according to claim 1, wherein the carboxyalkylated, reduced polysaccharide isolated as a sodium salt does not contain an infrared absorption peak in the region of about 1650 cm^{-1} to about 1800 cm^{-1}.

9. (once amended) A method according to claim 1, wherein producing the carboxyalkylated reduced polysaccharide is achieved at a temperature of less than about 50 °C.

10. A method according to claim 9, wherein producing the carboxyalkylated reduced polysaccharide is achieved at a temperature of less than about 40 °C.

11. A method according to claim 1, wherein the iron oxide is superparamagnetic.

12. A reduced polysaccharide iron oxide complex produced according to the method of claim 1, wherein the produced complex is stable at a temperature of at least 100 °C.

13. A reduced carboxyalkylated polysaccharide iron oxide complex wherein the reduced complex is stable at a temperature of about 121 °C.

14. A reduced polysaccharide iron oxide complex according to claim 13, wherein the reduced complex is stable at a temperature of at least about 121 °C for a period of time effective to sterilize the complex.

15. A reduced polysaccharide iron oxide complex according to claim 14, wherein the carboxyalkylated reduced polysaccharide is selected from the group consisting of a carboxymethyl, carboxyethyl and carboxypropyl reduced polysaccharide.

16. A reduced polysaccharide iron oxide complex according to claim 15, wherein the reduced polysaccharide is a reduced dextran.

17. A reduced polysaccharide iron oxide complex according to claim 15, wherein the carboxyalkylated reduced dextran is a carboxymethyl reduced dextran.

18. A reduced polysaccharide iron oxide complex according to claim 16, wherein the carboxyalkylated reduced dextran comprises at least about 750 micromole of carboxyl groups per gram of polysaccharide.

19. A reduced polysaccharide iron oxide complex according to claim 18, wherein the carboxyalkylated reduced dextran comprises at least about 900 micromole of carboxyl groups per gram of polysaccharide.

20. A reduced polysaccharide iron oxide complex according to claim 19, wherein the carboxyalkylated reduced dextran comprises at least about 1100 micromole of carboxyl groups per gram of polysaccharide.

21. A reduced polysaccharide iron oxide complex according to claim 20, wherein the carboxyalkylated reduced dextran comprises less than about 1500 micromole of carboxyl groups per gram of polysaccharide wherein said complex does not form substantial particulates.

23. A method of providing a hematinic agent for treating a subject deficient in iron according to claim 1, consisting of the steps of:

formulating a composition which is a carboxymethylated reduced polysaccharide ultrasmall iron oxide complex; and

terminally sterilizing the composition by autoclaving.

24. A method according to claim 22 or 23, having the further step of providing the autoclaved composition in a unit dosage.

25. A reduced carboxyalkylated polysaccharide iron oxide complex which is stable at a temperature of about 121 °C, wherein a sodium salt of the complex does not contain an infrared absorption peak in the region of about 1650

cm^{-1} to about 1800 cm^{-1}.

26. A reduced carboxyalkylated polysaccharide iron oxide complex according to claim 25, wherein the polysaccharide is carboxymethylated.

Following is a summary of the manner by which claims of the patent cover the approved product and the method of manufacturing the approved product. Claim 1, which is directed to a method of providing an iron oxide complex for administration to a mammalian subject, covers the method of manufacturing the approved product. The approved product is a carboxymethylated reduced dextran superparamagnetic iron oxide complex that has been sterilized by autoclaving. The limitations of claim 1 require producing a carboxyalkylated reduced polysaccharide iron oxide complex (which characterizes the approved product) and sterilizing the complex by autoclaving. Accordingly, claim 1 covers the method of manufacturing the approved product.

Claims 2-11 and 23-24, which depend on claim 1, provide further limitations on the method of manufacturing described by claim 1, including limitations which specify the nature of the reduced polysaccharide, the nature of individual steps utilized in manufacturing the carboxyalkylated reduced polysaccharide iron oxide complex, the nature of providing the carboxyalkylated reduced polysaccharide iron oxide complex for administration, and the nature of certain physicochemical properties of the carboxyalkylated reduced polysaccharide iron oxide complex.

Claim 13 is directed to a reduced carboxyalkylated polysaccharide iron oxide complex that is stable at a temperature of about 121 °C. Because the approved product is an autoclavable superparamagnetic carboxymethylated reduced dextran iron oxide complex, the language of claim 13 covers the approved product. Claims 14-21 and 25-26, which depend on claim 13, require further limitations regarding the nature of the thermal stability of the carboxyalkylated reduced polysaccharide iron oxide complex, the nature and quantity of the carboxyalkyl substituent of the carboxyalkylated reduced polysaccharide iron oxide complex, the nature of the polysaccharide of the carboxyalkylated reduced polysaccharide iron oxide complex, and the nature of certain physicochemical properties of the carboxyalkylated reduced polysaccharide iron oxide complex.

Thus, U.S. Patent 6,599,498 claims the approved product as well as methods of manufacturing the approved product.

(10) **A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:**

(i) For a patent claiming a human drug, antibiotic, or human biological product:

(A) The effective date of the investigational new drug (IND) application and the IND number;
(B) The date on which a new drug application (NDA) application or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and
(C) The date on which the NDA was approved or the Product License issued.

The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(i)(A) The first Investigational New Drug Application (IND 58,254) was filed **May 5, 1999** and became effective on **June 4, 1999**. The second Investigational New Drug Application (IND 62,745) was filed on June 14, 2001 and became effective on July 14, 2001.

(B) The New Drug Application (NDA 22-180) for the AMAG Product was initially submitted to the FDA on **December 18, 2007**, and

(C) the New Drug Application (NDA 22-180) was approved on **June 30, 2009**.

(11) **A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities.**

A brief description of the significant activities undertaken by marketing applicant during the applicable regulatory review period is attached hereto as Attachment E and is a reverse chronological synopsis of the major communications between Applicant and the FDA during the applicable regulatory review period.

Applicant reserves the right to supplement the information contained in Attachment E with materials from which it was derived or other evidence related to Applicant's conduct in obtaining the approval of the AMAG Product. *See, e.g.*, 21 C.F.R. § 60.32.

(12) **A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined.**

Applicant is of the opinion that U.S. Patent 6,599,498 is eligible for extension under 35 U.S.C. § 156 because it satisfies all of the requirements for such extension as follows:

(a) **35 U.S.C. § 156(a); 37 C.F.R. § 1.720(a)**

U.S. Patent 6,599,498 claims (i) a product, and (ii) a method of manufacturing a product, as defined in 37 C.F.R. § 1.710(a).

(b) **35 U.S.C. § 156(a)(1); 37 C.F.R. § 1.720(g)**

The term of U.S. Patent 6,599,498 has not expired before submission of this application, which is believed to be in compliance with 37 C.F.R. § 1.741.

(c) **35 U.S.C. § 156(a)(2); 37 C.F.R. § 1.720(b)**

The term of U.S. Patent 6,599,498 has never been extended under 35 U.S.C. § 156(e)(1).

(d) **35 U.S.C. § 156(a)(3); 37 C.F.R. § 1.730**

The application for extension is submitted by a registered practitioner on behalf of the owner of record in accordance with the requirement of 35 U.S.C. §156(d) and the rules of the U.S. Patent and Trademark Office, Proof that Applicant is the owner of record is provided by the following: (1) a copy of the Assignment of U.S. Patent 6,599,498 to Advanced Magnetics, Inc. on March 6, 2000 and a copy of the Recordation of same with the U.S. Patent and Trademark Office on March 8, 2000 (Attachments F and G) and (2) a copy of the Change of Name as filed with the Delaware Secretary of State showing Advanced Magnetics, Inc. changed its name to AMAG Pharmaceuticals, Inc. on July 24, 2007 and a copy of the Recordation of same with the U.S. Patent and Trademark Office on January 29, 2008. (Attachments H and I). Proof that the registered practitioner is authorized to act on behalf of the patent owner is provided by a copy of the power of attorney filed with

the U.S. Patent and Trademark Office in connection with U.S. Pat. No. 6,599,498 on October 27, 2008 (Attachment J).

(e) **35 U.S.C. § 156(a)(4); 37 C.F.R. § 1.720(d)**

The AMAG Product has been subject to a regulatory review period as defined in 35 U.S.C. § 156(g) before its commercial marketing or use.

(f) **35 U.S.C. § 156(a)(5)(A); 37 C.F.R. § 1.720(e)(1)**

The commercial marketing or use of the AMAG Product after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug and Cosmetics Act (21 U.S.C. § 355) under which such regulatory review period occurred.

(g) **35 U.S.C. § 156 (c)(4); 37 C.F.R. § 1.720(h)**

No other patent has been extended for the same regulatory review period for the AMAG Product.

(h) **35 U.S.C. § 156(d)(1); 37 C.F.R. §1.720(f)**

The application is submitted within the permitted 60 day period beginning on the date the product first received permission for commercial marketing or use.

(i) The length of extension of the patent term of U.S. Patent 6,599,498 claimed by applicant is **1209** days. The length of extension was determined pursuant to 35 U.S.C. § 156(g)(1) and 37 C.F.R. § 1.775(c) as follows:

(i) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) began **June 4, 1999** and ended **June 30, 2009**, a total of **3681** days, which is the sum of (ii) and (iii) below;

(ii) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), the IND period, began on **June 4, 1999** and ended on **December 18, 2007,** which is **3120** days.

(iii) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), the Application Period, began on **December 18, 2007** and ended **June 30, 2009,** which is **561** days.

(j) The regulatory review period upon which the period of extension is calculated is the entire regulatory review period as determined in subparagraph 12(i)(i) hereof (**3681** days) less:

(i) The number of days in the regulatory review period which were on or before the date on which the patent issued (**July 29, 2003**), which is **1517** days, and

(ii) The number of days during which applicant did not act with due diligence, which is **zero (0) days**, and

(iii) One-half the number of days determined in subparagraph 12(i)(ii) hereof (**3120**) after subtracting therefrom the number of days of subparagraphs (12)(j)(i) and (j)(ii) hereof (**1517** days in total), or **801.5** days (half days are ignored for purposes of subtraction), which totals **1363** days.

(k) The number of days as determined in subparagraph 12(j)(iii) hereof (**1363** days) when added to the original term of the patent would result in the date **December 1, 2023.**

(l) Fourteen (14) years when added to the date of the NDA approval **(June 30, 2009)** would result in the date **June 30, 2023.**

(m) The earliest date as determined in paragraphs 12(k) and 12(l) is **June 30, 2023.**

(n) The issuance of the original exemption occurred after September 24, 1984. Five (5) years when added to the original expiration date of the patent **(March 8, 2020)** would result in the date **March 8, 2025**.

(o) The earlier date as determined in paragraphs (m) and (n) is **June 30, 2023**. The

patent is currently set to expire on March 8, 2020. Therefore, the length of extension of patent term claimed by applicant is **1209** days.

(13) **A statement that the Applicant acknowledges a duty to disclose to the Director of the U.S. Patent and Trademark Office and to the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.**

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) **Prescribed Fee:**

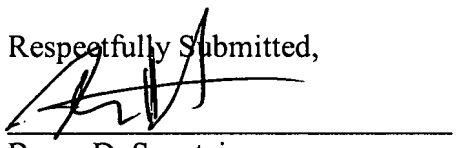
The prescribed fee pursuant to 37 C.F.R. § 1.20(j) for receiving and acting upon this application is to be charged to the Deposit Account of Applicant as authorized in the attached letter.

(15) **The name, address and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed:**

Bruce D. Sunstein
Sunstein Kann Murphy & Timbers LLP
Boston, MA 02110
(617) 443-9292 (ext. 211)
(617) 443-0004 (Fax)

Four copies of these application papers, certified as such, are being submitted herewith, in compliance with 37 C.F.R. § 1.740(b) and as suggested by MPEP § 2753.

Respectfully Submitted,



Bruce D. Sunstein
Reg. No. 27,234
Attorney for Applicant
Sunstein Kann Murphy & Timbers LLP
Boston, MA 02110
(617) 443-9292 (ext. 366)
(617) 443-0004 (Fax)

01275/00700 940305.1

Attachment A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

NDA 22-180

NDA APPROVAL

AMAG Pharmaceuticals, Inc
Attention: Mohammed Salem, Ph.D., RAC
100 Hayden Avenue
Lexington, MA 02421

Dear Dr. Salem:

Please refer to your new drug application (NDA) dated December 18, 2007, received December 19, 2007, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Feraheme™ (ferumoxytol) Injection.

We acknowledge receipt of your submissions dated February 27, April 3, 10, 14 and 28, May 20, June 5 and 23, July 14, 16 and 24, August 4, 5 and 7, September 3 (2), 5, 22 (2), 23, 24 and 25, October 1, 3 and 30, December 17, 2008; January 7, February 10, March 20 and 30, April 8, 14 and 29 (2), May 5 and 26, June 4, 9, 10, 16 and 18, 2009.

The April 29, 2009, submission constituted a complete response to our December 22, 2008, action letter.

This new drug application provides for the use of Feraheme™ (ferumoxytol) Injection for the treatment of iron deficiency anemia in adult patients with chronic kidney disease (CKD).

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

PEDIATRIC RESEARCH EQUITY ACT (PREA)

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for ages 0 to < 2 years and deferring pediatric studies for ages 2 to < 18 years for this application.

Your deferred pediatric studies required under section 2 of the Pediatric Research Equity Act (PREA) are considered required postmarketing requirements. The statuses of these postmarketing studies shall be reported annually according to 21 CFR 314.81. These requirements are listed below.

1. To conduct a clinical trial in pediatric patients aged 2 to < 18 years who have iron deficiency anemia and who are receiving either hemodialysis or peritoneal dialysis. In addition to any

other items, the trial will obtain pharmacokinetic (PK), pharmacodynamic (PD) and safety data from at least 50 patients exposed to ferumoxytol. In this trial, patients will be randomized to oral iron (25 patients) or one of two dose ferumoxytol dose regimens (25 patients in each dose cohort). Endpoints will consist of PK, PD, comparisons of hemoglobin changes and safety summaries.

The timetable you submitted on June 9, 2009, states that you will conduct this study according to the following timetable:

Final clinical protocol submission date:	December 2009
Clinical trial completion date:	April 2013
Final trial report submission date:	October 2013

2. To conduct a clinical trial in pediatric patients aged 2 to < 18 years who have iron deficiency anemia and chronic kidney disease that does not require dialysis. In addition to any other items, the trial will obtain pharmacokinetic (PK), pharmacodynamic (PD) and safety data from at least 50 patients exposed to ferumoxytol. In this trial, patients will be randomized to oral iron (25 patients) or one of two dose ferumoxytol dose regimens (25 patients in each dose cohort). Endpoints will consist of PK, PD, comparisons of hemoglobin changes and safety summaries.

   The timetable you submitted on June 9, 2009, states that you will conduct this study according to the following timetable:

Final clinical protocol submission date:	December 2009
Clinical trial completion date:	April 2013
Final trial report submission date:	October 2013

Submit final study reports to this NDA. For administrative purposes, all submissions related to these pediatric postmarketing study requirements must be clearly designated "**Pediatric Study Requirements**".

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, please submit the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format as described at http://www.fda.gov/oc/datacouncil/spl.html that is identical to the enclosed labeling (text for the package insert) and/or submitted labeling (package insert submitted June 18, 2009). Upon receipt, we will transmit that version to the National Library of Medicine for public dissemination. For administrative purposes, please designate this submission, "SPL for approved NDA 22-180."

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels and/or submitted carton and immediate container labels as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005)*. Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Final Printed Carton and Container Labels for approved NDA 22-180.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert(s) to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert(s), at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see www.fda.gov/cder/ddmac.

Please submit one market package of the drug product when it is available.

LETTERS TO HEALTH CARE PROFESSIONALS

If you issue a letter communicating important safety related information about this drug product (i.e., a "Dear Health Care Professional" letter), we request that you submit an electronic copy of the letter to both this NDA and to the following address:

MedWatch
Food and Drug Administration
Suite 12B05
5600 Fishers Lane
Rockville, MD 20857

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

If you have any questions, call Hyon-Zu Lee, Pharm.D., Regulatory Project Manager, at 301-796-2050.

Sincerely,

{See appended electronic signature page}

Rafel Dwaine Rieves, M.D.
Director
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Feraheme safely and effectively. See full prescribing information for Feraheme.

Feraheme™ (ferumoxytol) Injection
For Intravenous (IV) use
Initial U.S. Approval: XXXX

INDICATIONS AND USAGE

Feraheme is an iron replacement product indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease (CKD). *(1)*

DOSAGE AND ADMINISTRATION

- The recommended dose of Feraheme is an initial 510 mg intravenous injection followed by a second 510 mg intravenous injection 3 to 8 days later.
- Administer Feraheme as an undiluted intravenous injection delivered at a rate of up to 1 mL/sec (30 mg/sec).
- The recommended Feraheme dose may be readministered to patients with persistent or recurrent iron deficiency anemia.

DOSAGE FORMS AND STRENGTHS

Feraheme (30 mg/mL) is available for intravenous injection in single use vials. Each vial contains 510 mg of elemental iron in 17 mL.

CONTRAINDICATIONS

Evidence of iron overload. *(4)*
Known hypersensitivity to Feraheme or any of its components. *(4)*
Anemia not caused by iron deficiency. *(4)*

WARNINGS AND PRECAUTIONS

- Hypersensitivity Reactions: Observe for signs and symptoms of hypersensitivity for at least 30 minutes following the administration of Feraheme. *(5.1)*
- Hypotension: Feraheme may cause hypotension. Monitor for signs and symptoms of hypotension following the administration of Feraheme. *(5.2)*
- Iron Overload: Regularly monitor hematologic responses during Feraheme therapy. Do not administer Feraheme to patients with iron overload. *(5.3)*
- Magnetic Resonance Imaging: Feraheme can alter magnetic resonance imaging (MRI) studies. *(5.4)*

ADVERSE REACTIONS

The most common adverse reactions (≥ 2%) following the administration of Feraheme are diarrhea, nausea, dizziness, hypotension, constipation, and peripheral edema. *(6.1)*

To report SUSPECTED ADVERSE REACTIONS with Feraheme, contact AMAG Pharmaceuticals, Inc. at 1-877- 411-2510, or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION

Revised: Month/Year

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Feraheme™ (ferumoxytol) Injection is indicated for the treatment of iron deficiency anemia in adult patients with chronic kidney disease (CKD).

2 DOSAGE AND ADMINISTRATION

The recommended dose of Feraheme is an initial 510 mg intravenous injection followed by a second 510 mg intravenous injection 3 to 8 days later. Administer Feraheme as an undiluted intravenous injection delivered at a rate of up to 1 mL/sec (30 mg/sec). The dosage is expressed in terms of mg of elemental iron, with each mL of Feraheme containing 30 mg of elemental iron. Evaluate the hematologic response (hemoglobin, ferritin, iron and transferrin saturation) at least one month following the second Feraheme injection. The recommended Feraheme dose may be readministered to patients with persistent or recurrent iron deficiency anemia.

For patients receiving hemodialysis, administer Feraheme once the blood pressure is stable and the patient has completed at least one hour of hemodialysis. Monitor for signs and symptoms of hypotension following each Feraheme injection.

Inspect parenteral drug products visually for the absence of particulate matter and discoloration prior to administration.

3 DOSAGE FORMS AND STRENGTHS

Feraheme (30 mg/mL) is available for intravenous injection in single use vials. Each vial contains 510 mg of elemental iron in 17 mL.

4 CONTRAINDICATIONS

Feraheme is contraindicated in patients with:

- Evidence of iron overload
- Known hypersensitivity to Feraheme or any of its components
- Anemia not caused by iron deficiency

5 WARNINGS AND PRECAUTIONS

5.1 HYPERSENSITIVITY REACTIONS

Feraheme may cause serious hypersensitivity reactions, including anaphylaxis and/or anaphylactoid reactions. In clinical studies, serious hypersensitivity reactions were reported in 0.2% (3/1,726) of subjects receiving Feraheme. Other adverse reactions potentially associated with hypersensitivity (e.g., pruritus, rash, urticaria or wheezing) were reported in 3.7% (63/1,726) of these subjects. Observe patients for signs and symptoms of hypersensitivity for at least 30 minutes following Feraheme injection and only administer the drug when personnel and therapies are readily available for the treatment of hypersensitivity reactions [*see Adverse Reactions (6.1)*].

5.2 HYPOTENSION

Hypotension may follow Feraheme administration. In clinical studies, hypotension was reported in 1.9% (33/1,726) of subjects, including three patients with serious hypotensive reactions. Monitor patients for signs and symptoms of hypotension following Feraheme administration [*see Dosage and Administration (2)* and *Warnings and Precautions (5.1)*].

5.3 IRON OVERLOAD

Excessive therapy with parenteral iron can lead to excess storage of iron with the possibility of iatrogenic hemosiderosis. Regularly monitor the hematologic response during parenteral iron therapy [*see Dosage and Administration (2)*]. Do not administer Feraheme to patients with iron overload [*see Contraindications (4)*].

In the 24 hours following administration of Feraheme, laboratory assays may overestimate serum iron and transferrin bound iron by also measuring the iron in the Feraheme complex.

5.4 MAGNETIC RESONANCE (MR) IMAGING

Administration of Feraheme may transiently affect the diagnostic ability of MR imaging. Anticipated MR imaging studies should be conducted prior to the administration of Feraheme. Alteration of MR imaging studies may persist for up to 3 months following the last Feraheme dose. If MR imaging is required within 3 months after Feraheme administration, use T1- or proton density-weighted MR pulse sequences to minimize the Feraheme effects; MR imaging using T2-weighted pulse sequences should not be performed earlier than 4 weeks after the administration of Feraheme. Maximum alteration of vascular MR imaging is anticipated to be evident for 1 – 2 days following Feraheme administration [*see Clinical Pharmacology (12.3)*].

Feraheme will not interfere with X-ray, computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasound or nuclear medicine imaging.

6 ADVERSE REACTIONS

Feraheme injection may cause serious hypersensitivity reactions and hypotension [*see Warnings and Precautions (5.1)(5.2)*].

In clinical studies, 1,726 subjects were exposed to Feraheme; 1,562 of these had CKD and 164 did not have CKD. Of these subjects 46% were male and the median age was 63 years (range of 18 to 96 years).

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug may not reflect the rates observed in practice.

6.1 ADVERSE REACTIONS IN CLINICAL STUDIES

Across the three randomized clinical trials [Trial 1, 2, and 3, *see Clinical Studies (14)*], a total of 605 patients were exposed to two injections of 510 mg of Feraheme and a total of 280 patients were exposed to 200 mg/day of oral iron for 21 days. Most patients received their second Feraheme injection 3 to 8 days after the first injection.

Adverse reactions related to Feraheme and reported by ≥ 1% of Feraheme-treated patients in the randomized clinical trials are listed in Table 1. Diarrhea (4.0%), constipation (2.1%) and hypertension (1.0%) have also been reported in Feraheme-treated patients.

Table 1: Adverse Reactions to Feraheme Reported in ≥1% of Patients with CKD

Adverse Reactions	Feraheme 2 x 510 mg (n = 605)	Oral Iron (n = 280)
Nausea	3.1%	7.5%
Dizziness	2.6%	1.8%
Hypotension	2.5%	0.4%
Peripheral Edema	2.0%	3.2%
Headache	1.8%	2.1%
Edema	1.5%	1.4%
Vomiting	1.5%	5.0%
Abdominal Pain	1.3%	1.4%
Chest Pain	1.3%	0.7%
Cough	1.3%	1.4%
Pruritus	1.2%	0.4%
Pyrexia	1.0%	0.7%
Back Pain	1.0%	0%
Muscle Spasms	1.0%	1.4%
Dyspnea	1.0%	1.1%
Rash	1.0%	0.4%

In clinical trials, adverse reactions leading to treatment discontinuation and occurring in ≥ 2 Feraheme-treated patients included hypotension, infusion site swelling, increased serum ferritin level, chest pain, diarrhea, dizziness, ecchymosis, pruritus, chronic renal failure, and urticaria.

Following completion of the controlled phase of the trials, 69 patients received two additional 510 mg intravenous injections of Feraheme (for a total cumulative dose of 2.04 g). Adverse reactions following this repeat Feraheme dosing were similar in character and frequency to those observed following the first two intravenous injections.

In a placebo-controlled, cross-over trial, 713 patients with CKD received a single 510 mg dose of Feraheme. Adverse reactions reported by these patients were similar in character and frequency to those observed in other clinical trials.

7 DRUG INTERACTIONS

Drug-drug interaction studies with Feraheme were not conducted. Feraheme may reduce the absorption of concomitantly administered oral iron preparations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no studies of Feraheme in pregnant women. In animal studies, Feraheme caused decreased fetal weights and fetal malformations at maternally toxic doses of 13-15 times the human dose. Use Feraheme during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In rats, administration of Feraheme at maternally toxic doses during organogenesis, i.e., daily doses approximately 2 times the recommended 510 mg human dose (on a mg/m^2 basis) for 12 days, caused a decrease in fetal weights. The cumulative animal exposure was approximately 13 times the human therapeutic course of 1.02 g (on a mg/m^2 basis). In rabbits, administration of Feraheme at maternally toxic doses during organogenesis, i.e., daily doses approximately 2 times the recommended 510 mg human dose (on a mg/m^2 basis) for 14 days, was associated with decreased fetal weights and external and/or soft tissue fetal malformations. The cumulative animal exposure was approximately 15 times the human therapeutic course of 1.02 g on a mg/m^2 basis [*see Nonclinical Toxicology (13.3)*].

8.3 Nursing Mothers

It is not known whether Feraheme is present in human milk. Because many drugs are excreted in human milk and because of the potential for adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to avoid Feraheme, taking into account the importance of Feraheme to the mother and the known benefits of nursing.

8.4 Pediatric Use

The safety and effectiveness of Feraheme in pediatric patients have not been established.

8.5 Geriatric Use

In controlled clinical trials, 330 patients ≥ 65 years of age were treated with Feraheme. No overall differences in safety and efficacy were observed between older and younger patients in these trials, but greater sensitivity of older individuals cannot be ruled out. In general, dose administration to an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy [*see Dosage and Administration (2) and Clinical Studies (14)*].

10 OVERDOSAGE

No data are available regarding overdosage of Feraheme in humans. Excessive dosages of Feraheme may lead to accumulation of iron in storage sites potentially leading to hemosiderosis. Do not administer Feraheme to patients with iron overload [*see Contraindications (4)*].

10.1 Nonclinical Data

No macroscopic or microscopic signs of toxicity and no changes in the clinical pathology data related to toxicity were observed following single intravenous doses of Feraheme up to 450 mg iron/kg in rats (approximately 10 times the recommended 510 mg human dose on a mg/m^2 basis) and in dogs (approximately 33 times the recommended 510 mg human dose on a mg/m^2 basis).

11 DESCRIPTION

Feraheme is a non-stoichiometric magnetite (superparamagnetic iron oxide) coated with polyglucose sorbitol carboxymethylether. The overall colloidal particle size is 17-31 nm in diameter. The chemical formula of Feraheme is $Fe_{5874}O_{8752}$-$C_{11719}H_{18682}O_{9933}Na_{414}$ with an apparent molecular weight of 750 kDa.

Feraheme injection is an aqueous colloidal product that is formulated with mannitol. It is a black to reddish brown liquid, and is provided in single use vials containing 510 mg of elemental iron. Each mL of the sterile colloidal solution of Feraheme injection contains 30 mg of elemental iron and 44 mg of mannitol, and has low bleomycin-detectable iron. The formulation is isotonic with an osmolality of 270-330 mOsm/kg. The product contains no preservatives, and has a pH of 6 to 8.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Feraheme consists of a superparamagnetic iron oxide that is coated with a carbohydrate shell, which helps to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the reticuloendothelial system macrophages of the liver, spleen and bone

marrow. The iron is released from the iron-carbohydrate complex within vesicles in the macrophages. Iron then either enters the intracellular storage iron pool (e.g., ferritin) or is transferred to plasma transferrin for transport to erythroid precursor cells for incorporation into hemoglobin.

12.2 Pharmacodynamics

Cardiac Electrophysiology
In a randomized, positive- and placebo-controlled, parallel-group study, healthy subjects received a supratherapeutic regimen of Feraheme (1.02 g given as two 510 mg doses within 24 hours), placebo or a single dose of 400 mg moxifloxacin (positive control). Results demonstrated no effect of Feraheme on QT interval durations. No clinically meaningful effect of Feraheme on heart rate was observed.

12.3 Pharmacokinetics

The pharmacokinetic (PK) behavior of Feraheme has been examined in healthy subjects and in patients with CKD stage 5D on hemodialysis. Feraheme exhibited dose-dependent, capacity-limited elimination from plasma with a half life of approximately 15 hours in humans. The clearance (CL) was decreased by increasing the dose of Feraheme. Volume of distribution (Vd) was consistent with plasma volume, and the mean maximum observed plasma concentration (C_{max}) and terminal half-life ($t_{1/2}$) values increased with dose. The estimated values of CL and Vd following two 510 mg doses of Feraheme administered intravenously within 24 hours were 69.1 mL/hr and 3.16 L, respectively. The C_{max} and time of maximum concentration (t_{max}) were 206 mcg/mL and 0.32 hr, respectively. Rate of infusion had no influence on Feraheme PK parameters. No gender differences in Feraheme PK parameters were observed. Feraheme is not removed by hemodialysis.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Feraheme was not tested for carcinogenic effects. In standard genotoxicity tests, Feraheme showed no evidence of mutagenic activity in an *in vitro* Ames test or clastogenic activity in either an *in vitro* chromosomal aberration assay or an *in vivo* micronucleus assay.

No adverse effects on fertility or general reproductive performance were noted in animal studies. Feraheme had no effect on male or female fertility or general reproductive performance in rats.

13.2 Animal Toxicology and Pharmacology

Animal studies demonstrate that the plasma half-life of Feraheme increased with increasing dose. The highest tissue concentrations of Feraheme were found in the liver, spleen, and central lymph node pool; administered radiolabeled Feraheme (^{59}Fe) was found in the red blood cell fraction by 24 hr. Studies with radiolabeled drug product demonstrated that renal elimination of the iron in Feraheme was insignificant, while the carbohydrate coating was significantly excreted in the urine and feces.

Repeat-dose toxicity studies with Feraheme up to 12 mg Fe/kg/day for 13 weeks in rats (cumulative exposure approximately 12 times the anticipated exposure of a human therapeutic course of 1.02 g of Feraheme on mg/m^2 basis) and dogs (cumulative exposure approximately 40 times the anticipated exposure of a human therapeutic course of 1.02 g of Feraheme on mg/m^2 basis) demonstrated dose-dependent decreases in body weight gain and food consumption, and increases in pigmentation intensity. No systemic toxicity or immunotoxicity was observed at the relevant clinical doses. Changes in red blood cell counts, hemoglobin and serum iron, increases in liver and spleen weight, and the accumulation of iron-positive pigmentation in various organs were observed as expected with the administration of iron-containing agents.

13.3 Reproductive and Developmental Toxicology

In rats, no maternal or fetal effects of Feraheme were observed at daily doses of 31.6 mg Fe/kg during organogenesis for 12 days, approximately 1 time the recommended human dose of 510 mg (on mg/m^2 basis). The cumulative animal exposure was approximately 5 times the human therapeutic course of 1.02 g (on a mg/m^2 basis). Administration of Feraheme during organogenesis at maternally toxic doses of 100 mg Fe/kg/day (daily exposure was approximately 2 times the recommended 510 mg human dose on a mg/m^2 basis) for 12 days (cumulative exposure was approximately 13 times the human therapeutic course of 1.02 g on a mg/m^2 basis) caused a decrease in fetal weights.

In rabbits, no maternal or fetal effects of Feraheme were observed at daily doses of 16.5 mg Fe/kg during organogenesis for 14 days, approximately 1 time the recommended human dose of 510 mg (on mg/m^2 basis). The cumulative animal exposure was approximately 7 times the human therapeutic course of 1.02 g (on a mg/m^2 basis). Administration of Feraheme during organogenesis at maternally toxic doses of 45 mg Fe/kg/day (daily exposure approximately 2 times the recommended 510 mg human dose on a mg/m^2 basis) for 14 days (cumulative exposure approximately 15 times the human therapeutic course of 1.02 g on a mg/m^2 basis) caused decreased fetal weights and external and/or soft tissue fetal malformations.

14 CLINICAL STUDIES

The safety and efficacy of Feraheme for the episodic treatment of iron deficiency anemia in patients with CKD were assessed in three randomized, open-label, controlled clinical trials (Trial 1, 2 and 3). These trials also included an uncontrolled, follow-up phase in which patients with persistent iron deficiency anemia could receive two additional 510 mg intravenous injections of Feraheme. The major efficacy results from the controlled phase of each study are shown in Table 2.

In all three trials, patients with CKD and iron deficiency anemia were randomized to treatment with Feraheme or oral iron. Feraheme was administered as two 510 mg intravenous single doses and oral iron (ferrous fumarate) was administered as a total daily dose of 200 mg elemental iron daily for 21 days. The major trial outcomes assessed the change in hemoglobin from baseline to Day 35. Trial 1 and 2 enrolled patients with non-dialysis dependent CKD and Trial 3 enrolled patients who were undergoing hemodialysis.

In Trial 1, the mean age of patients was 66 years (range, 23 to 95); 60% were female; 65% were Caucasian, 32% were Black, and 2% were other races. In the Feraheme and oral iron groups, 42% and 44% of patients, respectively, were receiving erythropoiesis stimulating agents (ESAs) at baseline.

In Trial 2, the mean age of patients was 65 years (range, 31 to 96); 61% were female; 58% were Caucasian, 35% were Black, and 7% were other races. In the Feraheme and oral iron groups, 36% and 43% of patients, respectively, were receiving ESAs at baseline.

In Trial 3, the mean age of patients was 60 years (range, 24 to 87); 43% were female; 34% were Caucasian, 59% were Black, and 7% were other races. All patients were receiving ESAs.

Table 2 shows the Baseline and mean change to Day 35 in hemoglobin (Hgb, g/dL), transferrin saturation (TSAT, %) and ferritin (ng/mL) in each treatment group for Trial 1, 2, and 3.

Table 2: Changes from Baseline to Day 35 in Hemoglobin, Transferrin Saturation and Ferritin (Intent to Treat Population)

	Trial 1 Non-Dialysis CKD		**Trial 2 Non-Dialysis CKD**		**Trial 3 CKD on Dialysis**	
ENDPOINT	Feraheme n = 226	Oral Iron n = 77	Feraheme n = 228	Oral Iron n = 76	Feraheme n = 114	Oral Iron n = 116
Baseline Hgb (mean ± SD, g/dL)	**9.9** ± 0.8	**9.9** ± 0.7	**10.0** ± 0.7	**10.0** ± 0.8	**10.6** ± 0.7	**10.7** ± 0.6
Hgb change from Baseline at Day 35 (mean ± SD, g/dL)	**1.2*** ± 1.3	**0.5** ± 1.0	**0.8*** ± 1.2	**0.2** ± 1.0	**1.0*** ± 1.1	**0.5** ± 1.1
Baseline TSAT (mean ± SD, %)	**9.8** ± 5.4	**10.4** ± 5.2	**11.3** ± 6.1	**10.1** ± 5.5	**15.7** ± 7.2	**15.9** ± 6.3
TSAT change from Baseline at Day 35 (mean ± SD, %)	**9.2** ± 9.4	**0.3** ± 4.7	**9.8** ± 9.2	**1.3** ± 6.4	**6.4** ± 12.6	**0.6** ± 8.3
Baseline ferritin (mean ± SD, ng/mL)	**123.7** ± 125.4	**146.2** ± 136.3	**146.1** ± 173.6	**143.5** ± 144.9	**340.5** ± 159.1	**357.6** ± 171.7
Ferritin change from Baseline at Day 35 (mean ± SD, ng/mL)	**300.7** ± 214.9	**0.3** ± 82.0	**381.7** ± 278.6	**6.9** ± 60.1	**233.9** ± 207.0	**-59.2** ± 106.2

* $p \leq 0.001$ for main efficacy endpoint

Following completion of the controlled phase of each of the Phase 3 trials, patients who were iron deficient and anemic could receive two additional 510 mg intravenous injections of

Feraheme for a total cumulative dose of 2.04 g. Overall, 69 patients received two additional 510 mg intravenous injections of Feraheme, and on Day 35 following these additional injections, the majority of these patients (70%) experienced an increase in hemoglobin and iron parameters (TSAT and ferritin). The mean change (±SD) in hemoglobin level from the retreatment baseline for patients with an increase in hemoglobin was 0.86 (± 0.68) g/dL and was 0.5 (± 0.8) g/dL for all patients.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Feraheme is available in single use vials in the following package sizes (Table 3).

Table 3: Feraheme Packaging Description

NDC Code	Dose / Total volume per vial	Vials / Carton
NDC 59338-775-01	510 mg/ 17 mL	1
NDC 59338-775-10	510 mg/ 17 mL	10

16.2 Stability and Storage

Store at controlled room temperature (20° to 25°C [68° to 77°F]). Excursions permitted to 15° – 30°C (59° – 86°F).

17 PATIENT COUNSELING INFORMATION

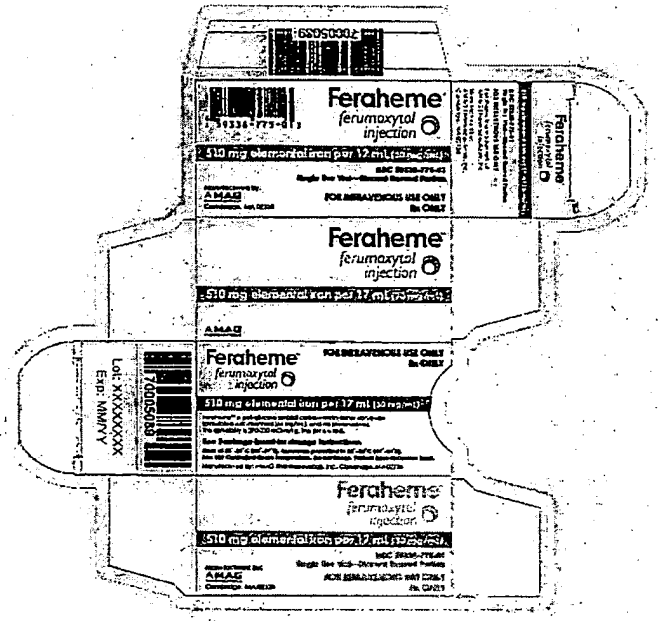
Prior to Feraheme administration:

- Question patients regarding any prior history of reactions to parenteral iron products.
- Advise patients of the risks associated with Feraheme.
- Advise patient to report any signs and symptoms of hypersensitivity that may develop during and following Feraheme administration, such as rash, itching, dizziness, lightheadedness, swelling and breathing problems [*see Warnings and Precautions (5)*].

Manufactured and Distributed by:

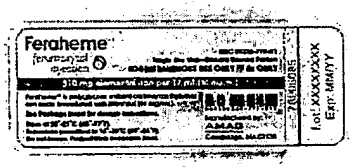
AMAG Pharmaceuticals, Inc.
Lexington, MA 02421

FINAL DRAFT PROOF	DATE: 15-June-09
Feraheme™ 510 mg/17 mL Carton (1 vial per carton)	Size: 1.4375 x 1.3125 x 2.75 inches (36.5125 x 33.3375 x 69.85 mm)
Part No.: 70005089	NDC Code: 59338-775-01
Copy: [redacted] PMS 377: [redacted] PMS 308: [redacted] PMS 185: [redacted] Die Line: [redacted] Varnish: [redacted]	AMAG



Feraheme
ferumoxytol
injection

FINAL DRAFT PROOF	DATE: 15-June -09
Feraheme™ 510 mg/17mL Vial (1 vial per carton)	Size: 3.5 x 1.25 inches (88.9 x 31.75 mm)
Part No.: 78005085	NDC Code: 59338-775-01
Copy PMS 377 PMS 368 PMS 185 Die Line Varnish	AMAG



FINAL DRAFT PROOF						DATE: 15-June -09
Feraheme™ 510 mg/17mL Carton (10 vials per carton)						Size: 6.25 x 2.5 x 2.625 inches (158.75 x 63.5 x 66.675 mm)
Part No.: 72005092			MDC Code: 59338-775-10			
2 Copy	PMS 377	PMS 308	PMS 185	Die Line	Varnish	AMAG

510 mg elemental iron per 17 mL (30 mg/mL)

Feraheme™
ferumoxytol injection

AMAG

72005092

Lot: XXXXXXXX
Exp: MM/YY

Feraheme™
ferumoxytol injection

510 mg elemental iron per 17 mL (30 mg/mL)

Manufactured by:
AMAG
Cambridge, MA 02138

FOR INTRAVENOUS USE ONLY Rx ONLY

Feraheme™
ferumoxytol injection

510 mg elemental iron per 17 mL (30 mg/mL)

NDC 59338-775-10
10 x 17 mL
Single Use Vials—Discard Unused Portion
FOR INTRAVENOUS USE ONLY
Feraheme is a trademark of AMAG Pharmaceuticals, Inc.
Manufactured by:
AMAG Pharmaceuticals, Inc.
Cambridge, MA 02138

Feraheme™
ferumoxytol injection

Manufactured by:
AMAG
Cambridge, MA 02138

510 mg elemental iron per 17 mL (30 mg/mL)

Feraheme™ is polyglucose sorbitol carboxymethylether iron oxide formulated with mannitol (44 mg/mL) and no preservatives. The osmolality is 270-330 mOsm/kg. The pH is 6 to 8.

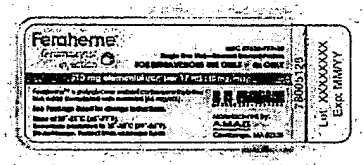
See Package Insert for dosage instructions.

Store at 20°-25°C (68°-77°F).
Excursions permitted to 15°-30°C (59°-86°F).
See USP Controlled Room Temperature.
Do not freeze. Protect from excessive heat.

FOR INTRAVENOUS USE ONLY
Rx ONLY

3 59338-775-10 5

FINAL DRAFT PROOF?	DATE: 15-June -09
Feraheme™ 510 mg/17mL Vial (10 vials per carton)	Size: 3.5 x 1.25 inches (88.9 x 31.75 mm)
Part No.: 78005126	NDC Code: 59338-775-10
Copy: PMS 377, PMS 308, PMS 185, Die Line, Varnish	AMAG PHARMACEUTICALS



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Rafel Rieves
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Attachment B

US006599498B1

(12) **United States Patent**
Groman et al.

(10) Patent No.: US 6,599,498 B1
(45) Date of Patent: Jul. 29, 2003

(54) **HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND CARBOHDRATE DERIVATIVES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/521,264

(22) Filed: Mar. 8, 2000

Related U.S. Application Data

(60) Provisional application No. 60/128,579, filed on Apr. 9, 1999.

(51) Int. Cl.[7] A61B 5/055; A61K 9/16; A61K 9/50; A61K 31/70; A61K 31/715; A01N 43/04

(52) U.S. Cl. 424/9.34; 424/9.35; 424/493; 514/54; 514/59

(58) Field of Search 424/9.3, 9.32, 424/9.35, 493; 514/54, 59

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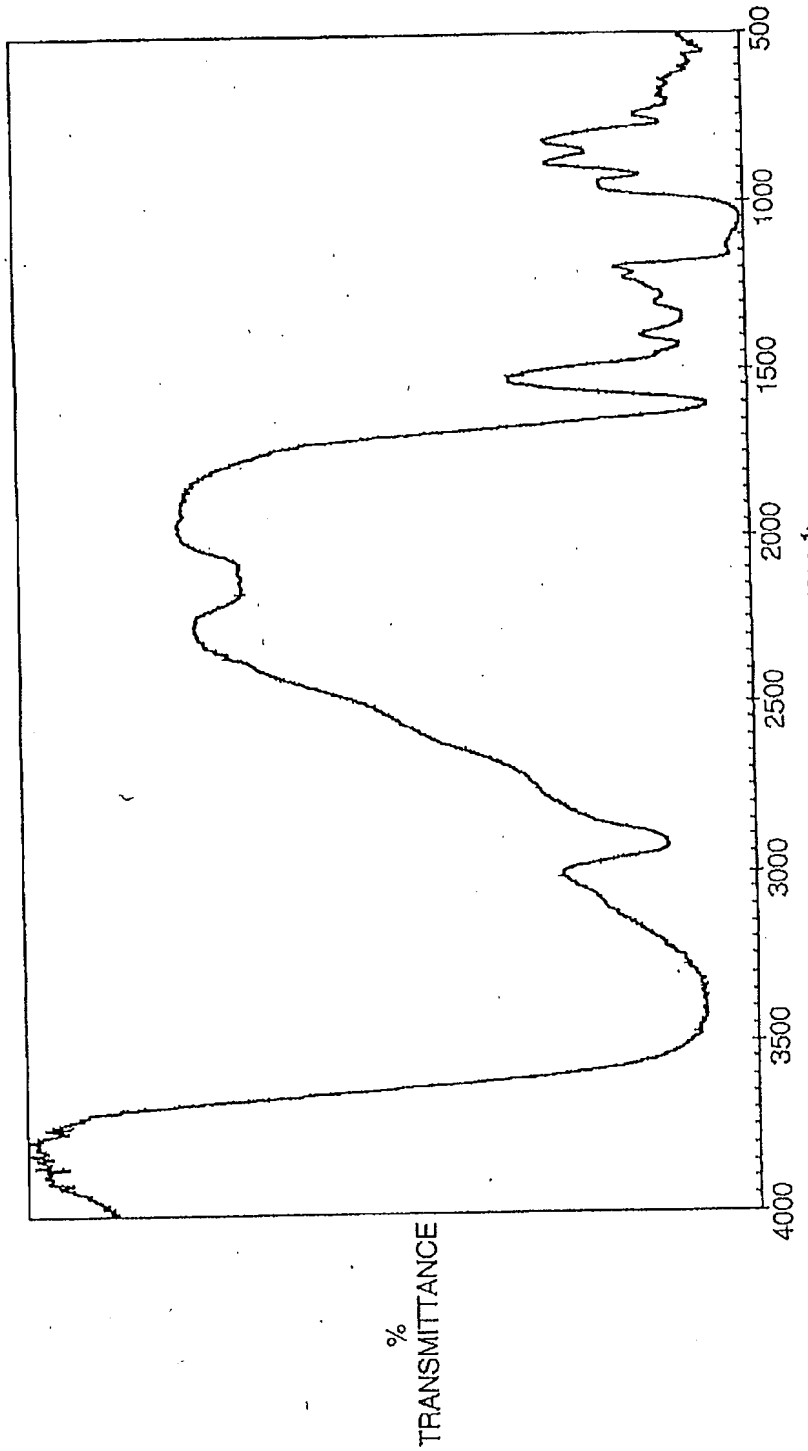
Primary Examiner—Sreeni Padmanabhan
(74) *Attorney, Agent, or Firm*—Bromberg & Sunstein LLP

(57) ABSTRACT

Compositions, methods of making the compositions, and methods of using the compositions are provided for an enhanced magnetic resonance imaging agent and a hematinic agent, the agents comprising carboxyalkylated reduced polysaccharides coated ultrasmall superparamagnetic iron oxides. Methods of use of the carboxymethyl reduced dextran as a plasma extender are provided.

26 Claims, 12 Drawing Sheets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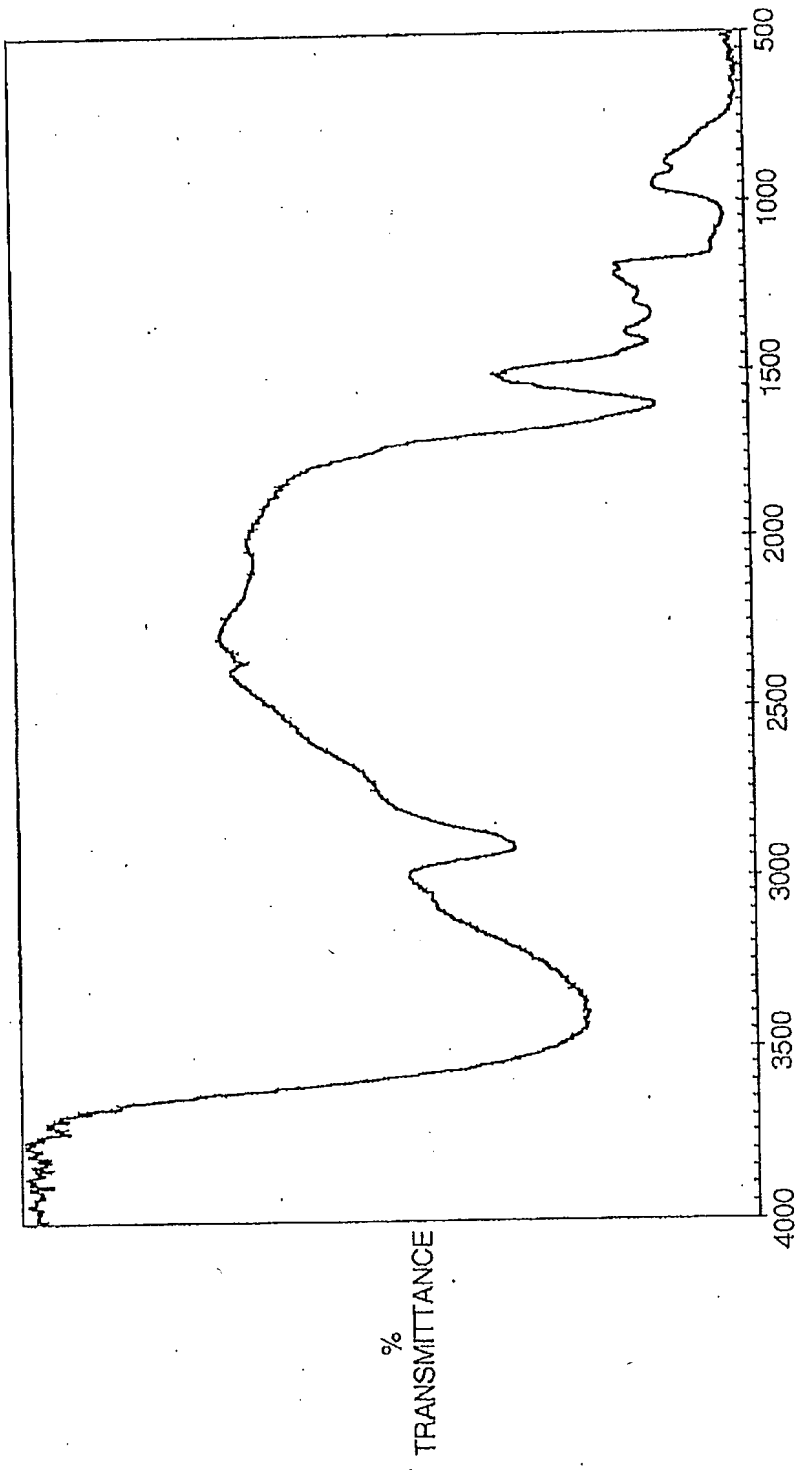
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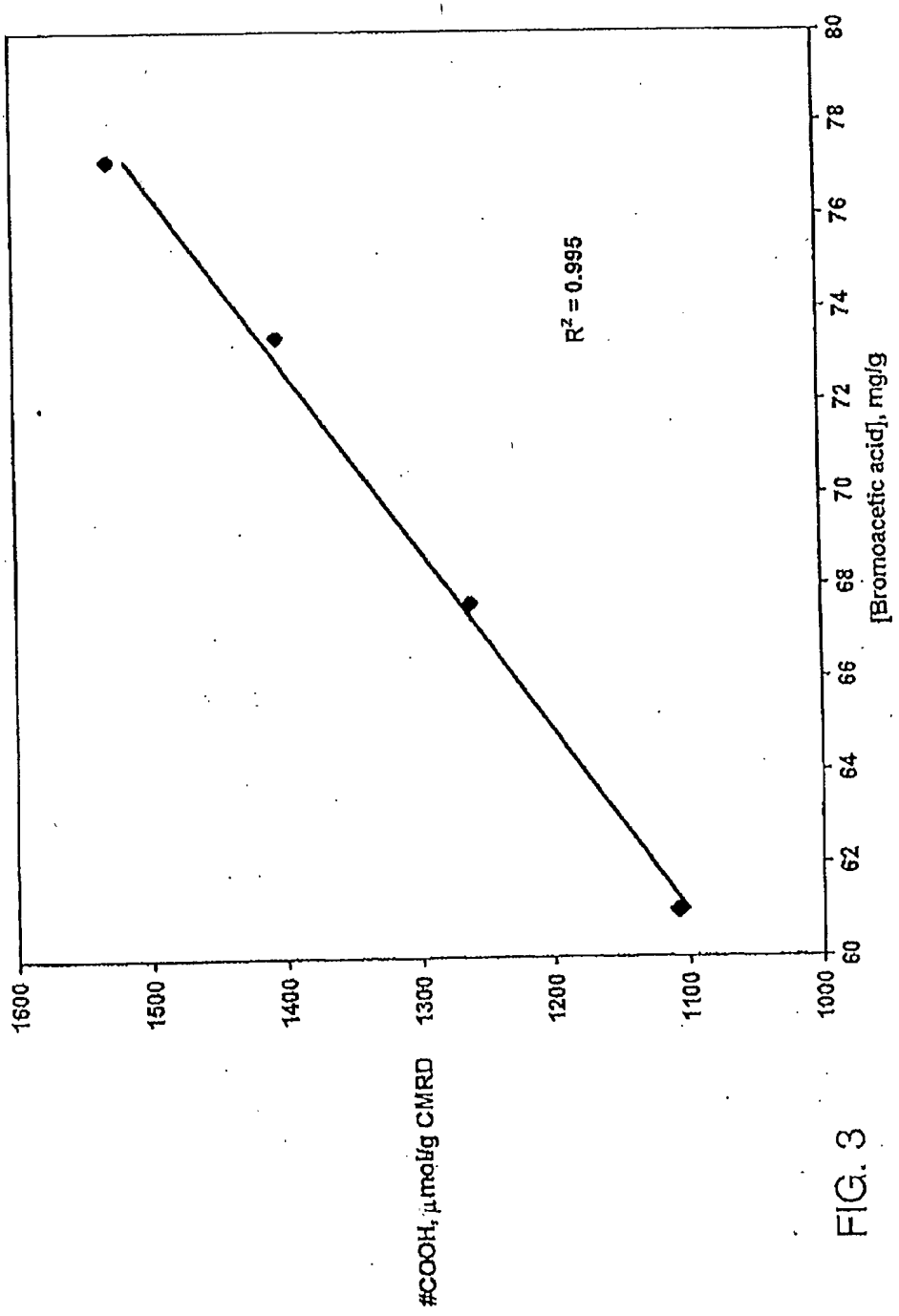
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76
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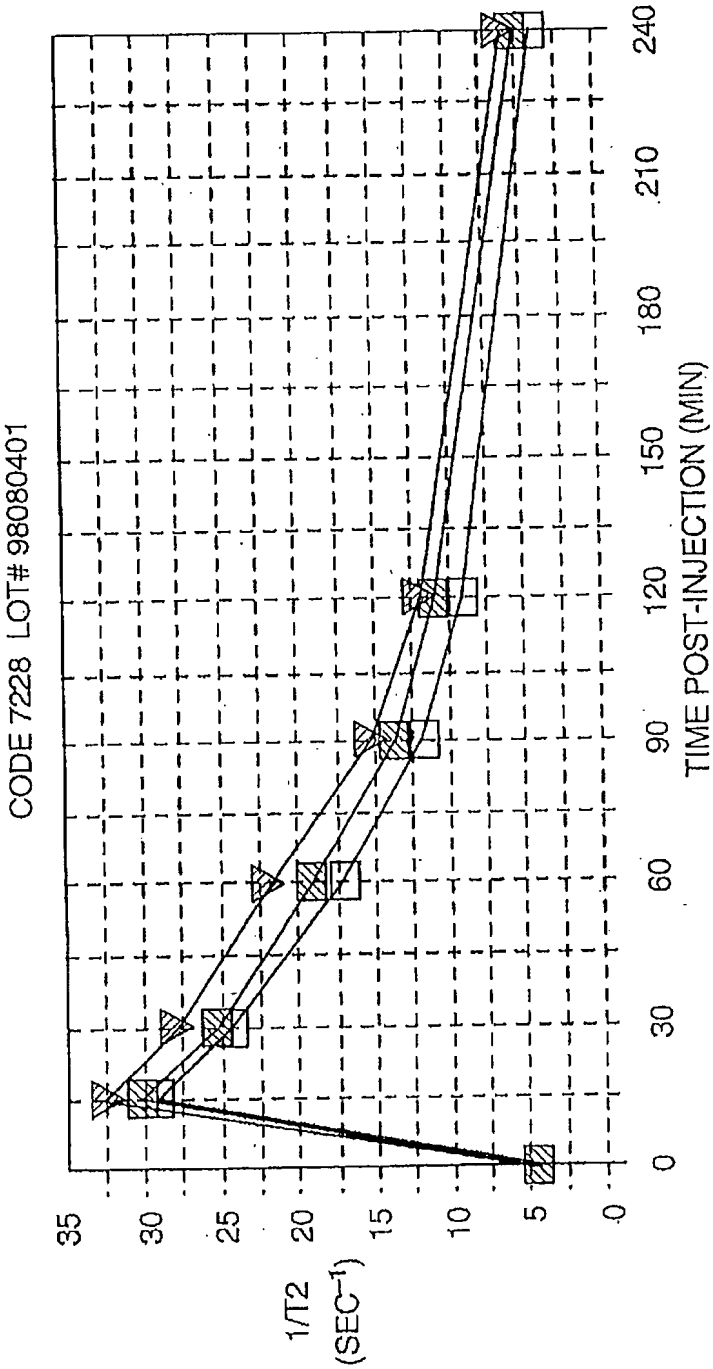
80

FIG. 3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30
35
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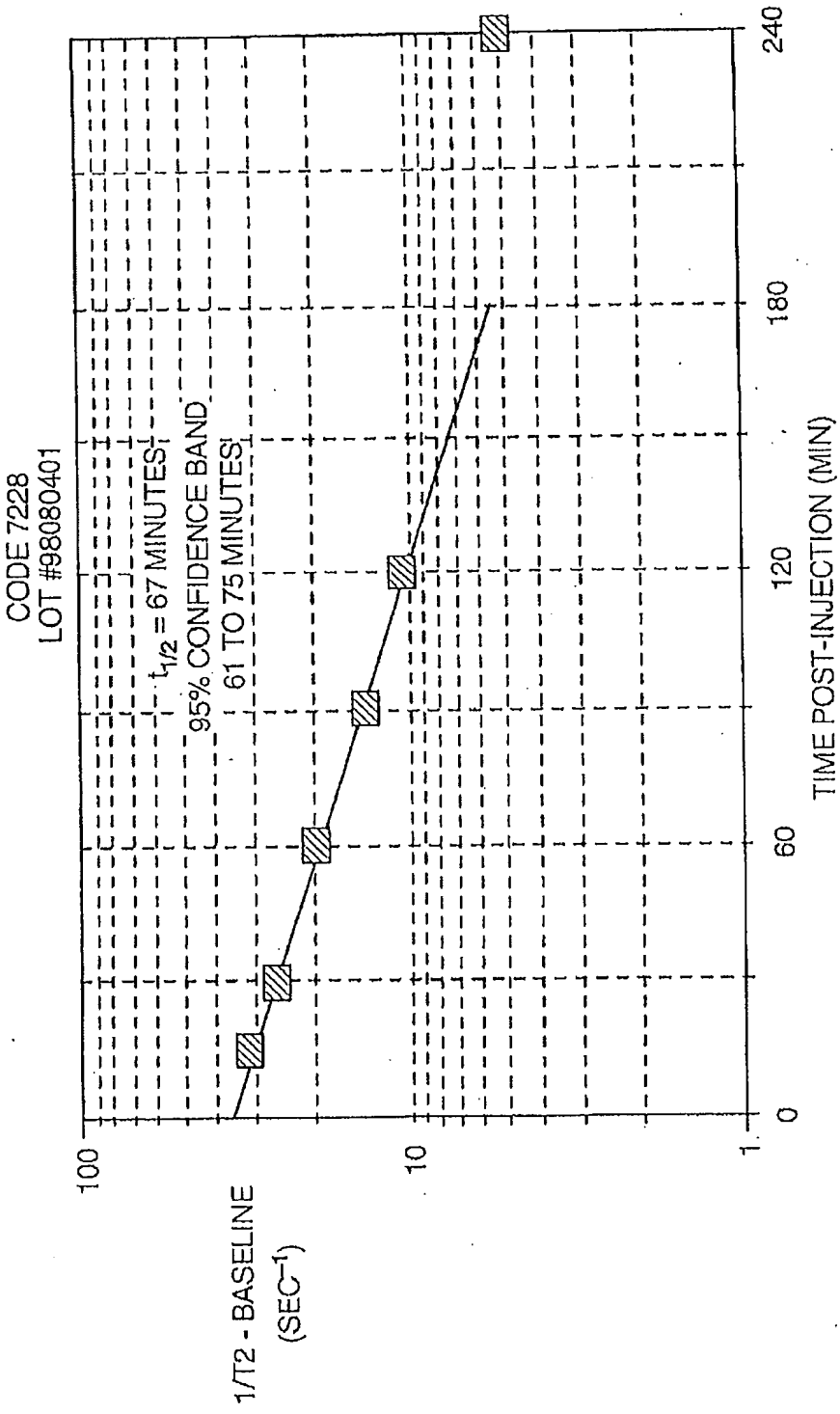
240
RAT #1
RAT #2
RAT #3

FIG. 4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61 TO 75 MINUTES
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(SEC^{-1})
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0
60
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180
240
TIME POST-INJECTION (MIN)

FIG. 5

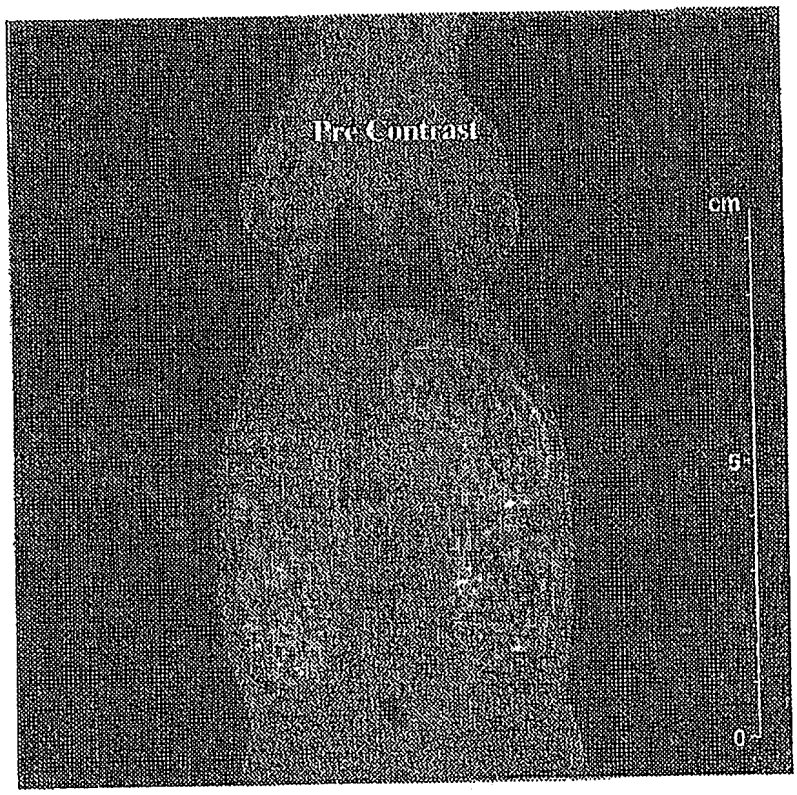


FIG. 6A

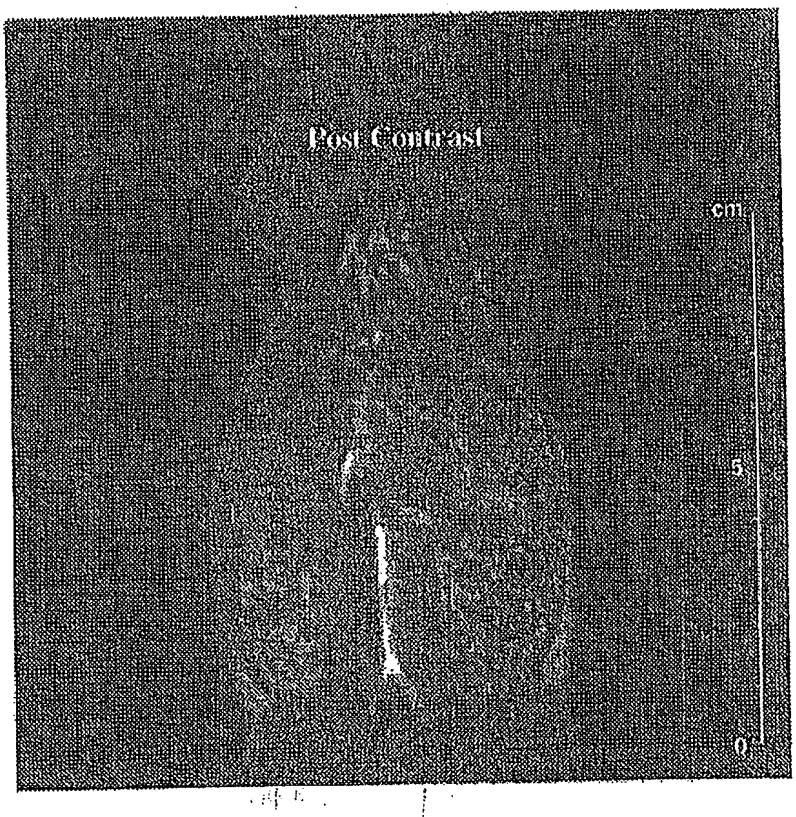


FIG. 6B

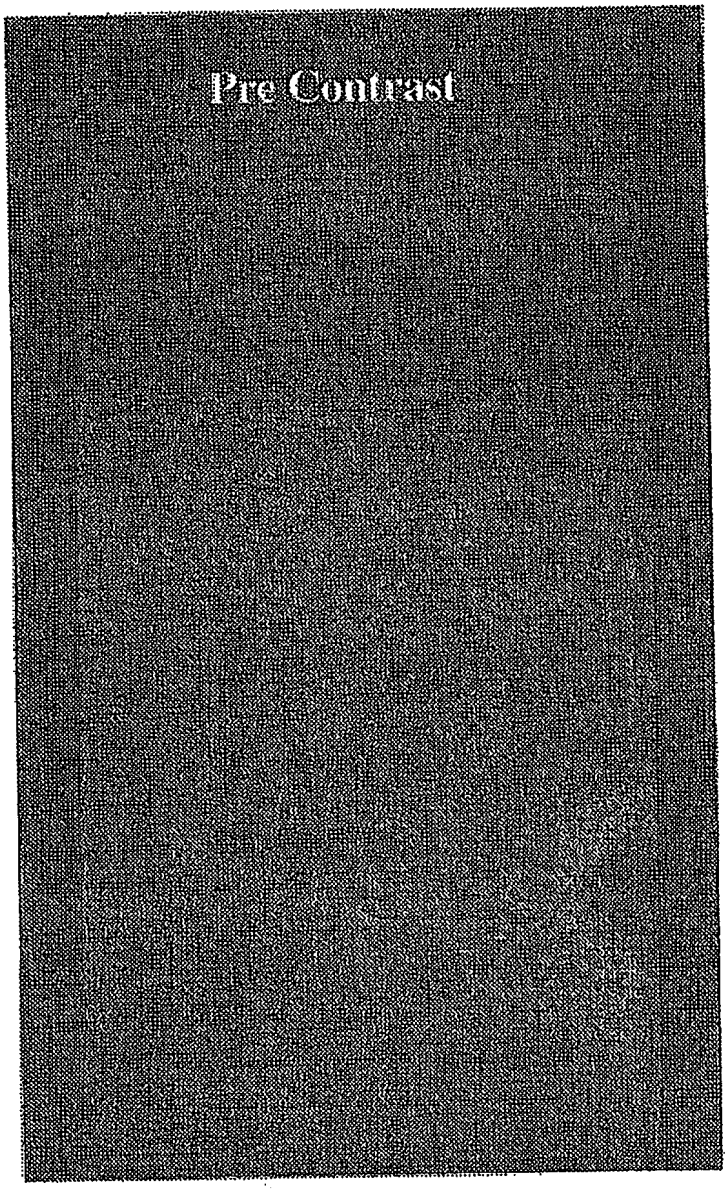


FIG. 7A

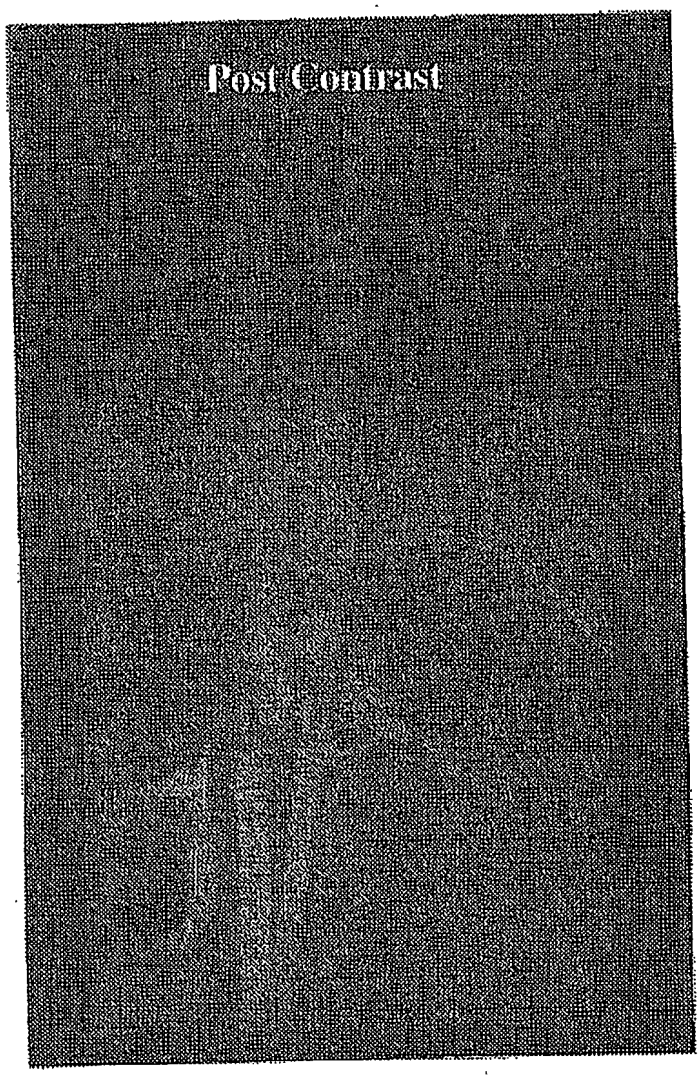


FIG. 7B

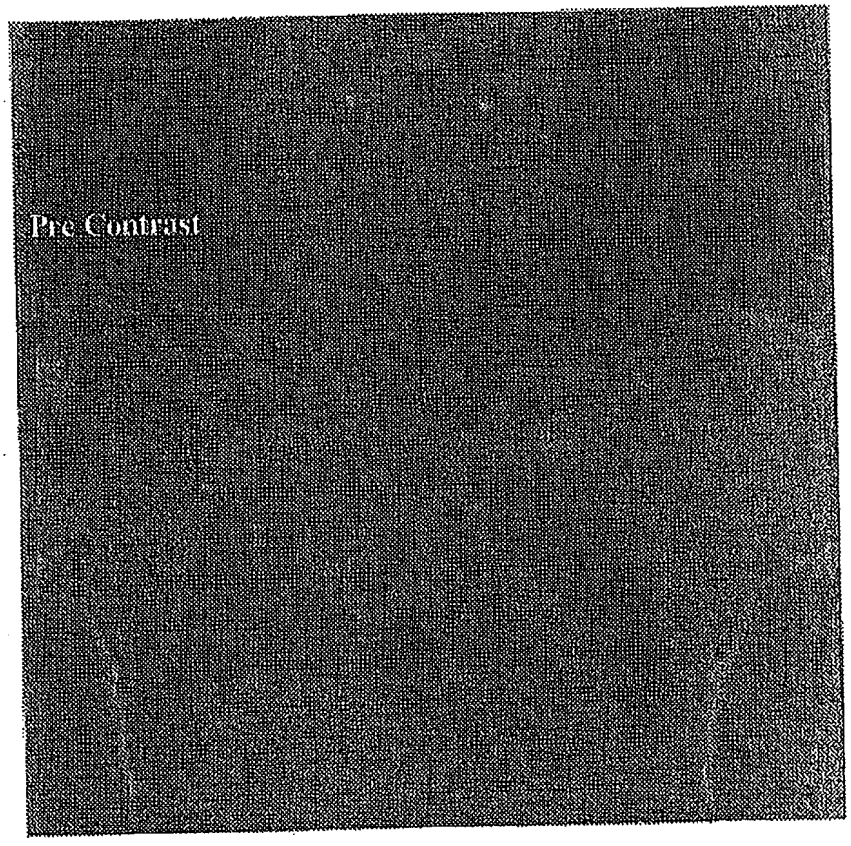


FIG. 8A

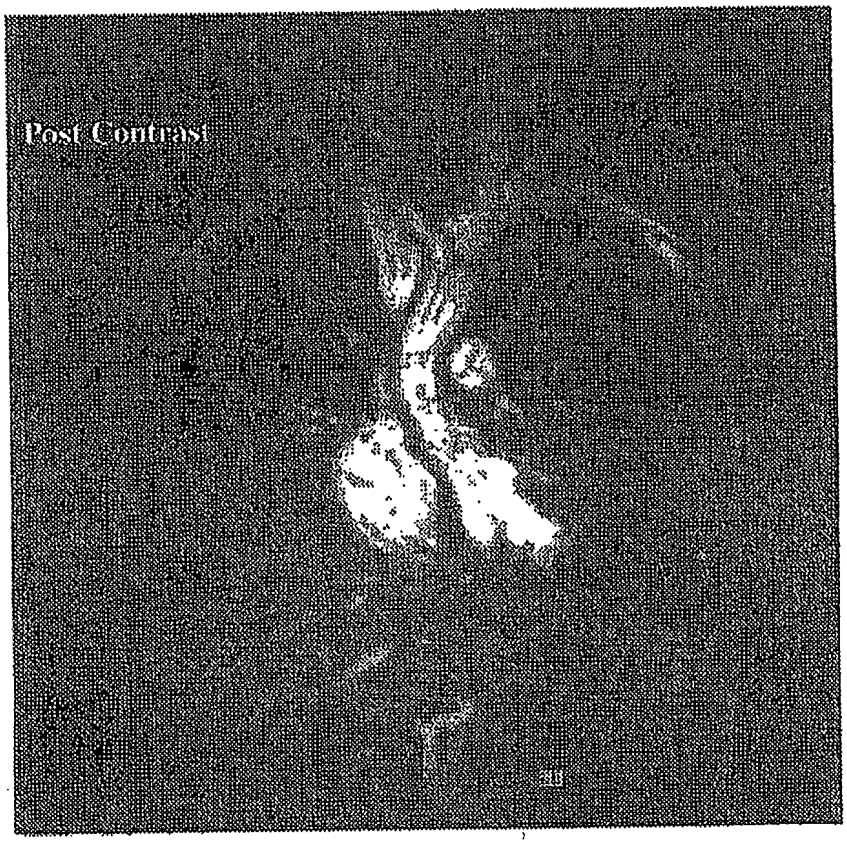
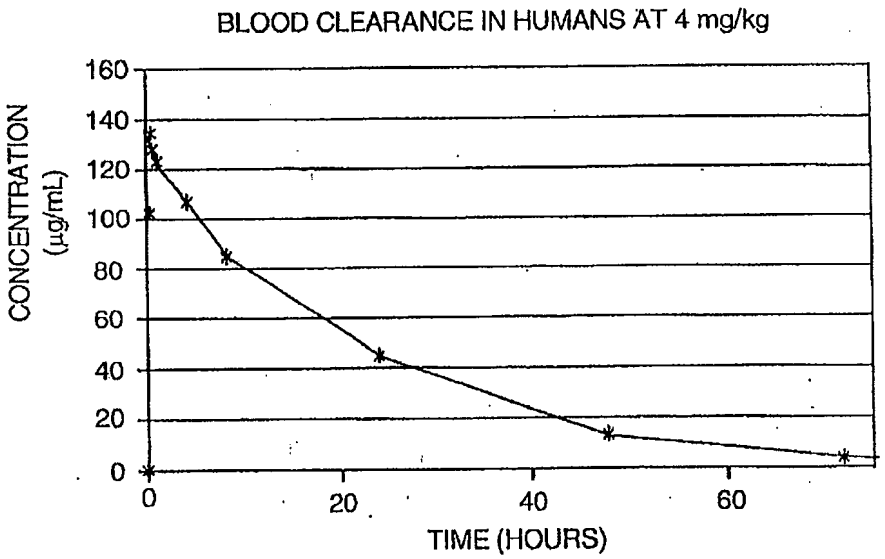


FIG. 8B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CONCENTRATION (µg/mL)
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100
80
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20
40
60
TIME (HOURS)

FIG. 9A

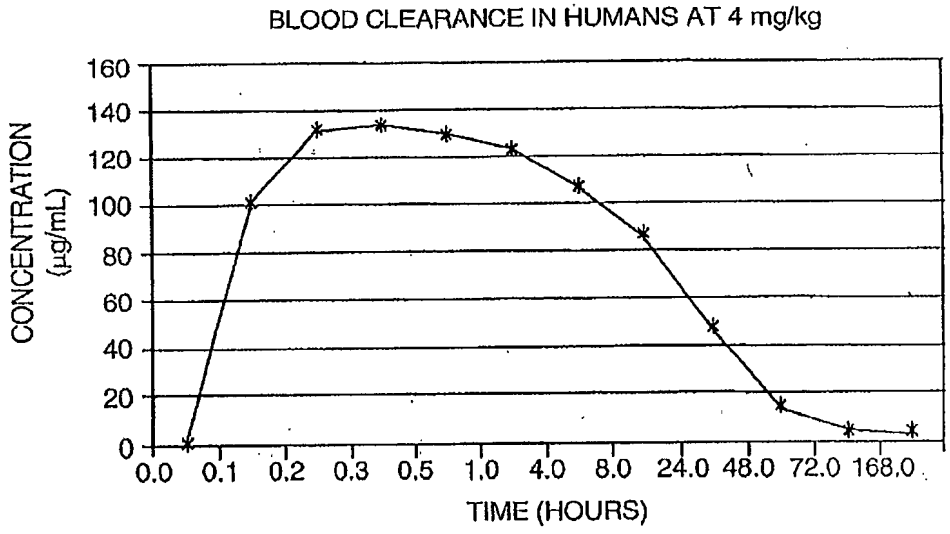

BLOOD CLEARANCE IN HUMANS AT 4 mg/kg
CONCENTRATION (µg/mL)
160
140
120
100
80
60
40
20
0
0.0
0.1
0.2
0.3
0.5
1.0
4.0
8.0
24.0
48.0
72.0
168.0
TIME (HOURS)

FIG. 9B

HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND CARBOHDRATE DERIVATIVES

RELATED APPLICATIONS

This application claims the benefit of Provisional Application No. 60/128,579, filed in the United States Patent and Trademark Office on Apr. 9, 1999, and which is hereby incorporated by reference herein.

TECHNICAL FIELD

The field relates to compositions which are carboxymethyl reduced polysaccharides, and methods for use as plasma extenders and for coating iron oxide particles, and compositions comprised of superparamagnetic and non-superparamagnetic iron oxides coated with a reduced polysaccharide or derivatized reduced polysaccharide, and methods for use as MRI contrast agents and hematinics.

BACKGROUND

Since the invention of magnetic resonance imaging (MRI), a parallel technology of injectable chemicals called contrast agents has developed. Contrast agents play an important role in the practice of medicine in that they help produce more useful MRI images for diagnostic purposes. In particular, two classes of imaging agents have been developed and adopted in clinical practice. These are: low molecular weight gadolinium complexes such as Magnavist®; and colloidal iron oxides. Neither of these two types of agents is ideal. Problems encountered with these agents are shown in Table 1, and include: expense of components; inefficiency of synthesis; loss of coating if sterilized by autoclaving; narrow range of organ uptake for purposes of imaging; side-effects; restriction of use to either first pass or equilibrium dosing; and others that are described herein. Agents that overcome these problems, and that combine the properties of these two types of contrast agents, are highly desirable.

TABLE 1

Comparison of ideal properties of MRI contrast agents with properties of low molecular weight gadolinium based contrast agents and colloidal iron oxides.

Properties of an ideal contrast agent	low molecular weight gadolinium	colloidal iron oxides
Low production costs: efficient synthesis	Yes	No
Autoclavable without excipients	Yes	No
T1 agent	Yes	Sometimes
T2 agent	No	Yes
Non toxic at vast excess	Yes	No
Imaging vascular compartment at early phase (as a bolus administration) and at a late stage (equilibrium phase).	No	No
Multiple administration in single examination	No	No
Image of multiple target organs	Yes	Sometimes
Bolus injection	Yes	No
Low volume of injection	No	No
Iron source for anemia	No	Yes

SUMMARY

An embodiment of the invention is a method of providing an iron oxide complex for administration to a mammal subject, the method comprising: producing a reduced polysaccharide iron oxide complex, and sterilizing the complex by autoclaving. In general, the reduced polysaccharide is a reduced polymer of glucose. An example of a reduced polymer of glucose is a reduced dextran. The reduced polysaccharide is produced through reaction of a polysaccharide with a reagent selected from the group consisting of a borohydride salt or hydrogen in the presence of a hydrogenation catalyst. In a further aspect of the method, the iron oxide is superparamagnetic.

Another preferred embodiment of the invention is a method of providing an iron oxide complex for administration to a mammalian subject, the method comprising: producing a derivatized reduced polysaccharide iron oxide complex, and sterilizing the complex by autoclaving. According to this method, producing the complex can include derivatizing a reduced polysaccharide by caboxyalkylation, for example, wherein the carboxyalkylation is a carboxymethylation. The term "derivatizing" and related terms (e.g. derivatives, derivatized, derivatization, etc) refer to the conventional sense of functionalization at the reactive sites of the composition. Further according to this method, the reduced polysaccharide can be a reduced dextran. The derivatized, reduced polysaccharide can be isolated as the sodium salt and does not contain an infrared absorption peak in the region of 1650–1800 cm^{-1}. In one aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than approximately 50° C. In another aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than approximately 40° C. In a further aspect of the method, the iron oxide is superparamagnetic.

In yet another embodiment, the invention provides a method of formulating an iron oxide complex coated with a reduced polysaccharide. This composition is for pharmacological use and the composition has decreased toxicity in comparison to an analogous iron oxide complex coated with native polysaccharide. The method of formulating such an iron oxide complex comprises: producing a reduced polysaccharide iron oxide complex, and sterilizing the complex by autoclaving. The formulation provides polysaccharide which was produced by reacting the polysaccharide with one of a reducing agent selected from the group consisting of a borohydride salt or hydrogen in the presence of an hydrogenation catalyst. The reduced polysaccharide iron oxide complex has such decreased toxicity. In a further aspect of the method, the iron oxide is superparamagnetic.

In yet another embodiment, the invention provides a method of formulating an iron oxide complex coated with a reduced derivatized polysaccharide. This composition is for pharmacological use and the composition has decreased toxicity in comparison to an analogous iron oxide complex coated with native derivatized polysaccharide. The method of formulating such an iron oxide complex comprises: producing a reduced derivatized polysaccharide iron oxide complex; and sterilizing the complex by autoclaving. According to this method, producing the complex can include derivatizing a reduced polysaccharide by carboxyalkylation, for example, wherein the carboxyalkylation is a carboxymethylation. Further according to this method, the reduced polysaccharide can be a reduced dextran. The derivatized, reduced polysaccharide can be isolated as the sodium salt and does not contain an infrared absorption peak in the region of 1650–1800 cm^{-1}. In one aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than

approximately 50° C. In another aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than approximately 40° C. In a further aspect of the method, the iron oxide is superparamagnetic.

Another embodiment of the invention provides a reduced derivatized polysaccharide iron oxide complex with T1 and T2 relaxation properties to allow contrast agent signal enhancement with T1 sequences and signal diminishment with T2 sequences. A further aspect of the embodiment is that the reduced derivatized polysaccharide iron oxide can be administered multiple times for sequential imaging in a single examination. Yet another aspect of the agent is that it can be used to image multiple organ systems including the vascular system, liver, spleen, bone marrow, and lymph nodes.

Another embodiment of the invention provides a reduced polysaccharide iron oxide complex for use as an intravenous iron supplement.

Another embodiment of the invention provides a reduced derivatized polysaccharide iron oxide complex for use as an intravenous iron supplement.

In yet a further embodiment, the invention provides an improved method of administering to a mammalian subject an autoclaved reduced polysaccharide iron oxide complex. The improved method of administration comprising: injection of an autoclaved reduced polysaccharide iron oxide complex in a volume of 15 ml or less. In another aspect of the embodiment the injected volume is injected as a bolus. In a further aspect of the method, the iron oxide is superparamagnetic. In a further aspect of the embodiment the injected volume provides improved image quality.

In yet a further embodiment, the invention provides an improved method of administering to a mammalian subject an autoclaved derivatized reduced polysaccharide iron oxide complex. The improved method of administration comprising: injection of an autoclaved reduced derivatized polysaccharide iron oxide complex in a volume of 15 ml or less. In another aspect of the embodiment the injected volume is injected as a bolus. In a further aspect of the method, the iron oxide is superparamagnetic. In a further aspect of the embodiment the injected volume provides improved image quality.

An embodiment of the invention provides an improved method of administering to a mammalian subject a reduced polysaccharide iron complex in a manner that the composition provides reduced toxicity, wherein the improvement comprises utilizing a reduced polysaccharide in formulation of the composition. In a further aspect of the embodiment, the iron oxide is superparamagnetic.

An embodiment of the invention provides an improved method of administering to a mammalian subject a reduced derivatized polysaccharide iron complex in a manner that the composition provides reduced toxicity, wherein the improvement comprises utilizing a reduced derivatized polysaccharide in formulation of the composition. In a further aspect of the embodiment, the iron oxide is superparamagnetic.

An embodiment of the invention provides a reduced polysaccharide iron oxide complex, wherein the reduced polysaccharide is derivatized, for example, the reduced derivatized polysaccharide is a carboxyalkyl polysaccharide. The carboxyalkyl is selected from the group consisting of carboxymethyl, carboxyethyl and carboxypropyl. Further, the reduced polysaccharide can be a reduced dextran, for example, the reduced dextran can be a reduced carboxymethyl dextran. A further aspect of this embodiment of the invention is that the level of derivatization of the reduced dextran is at least 750 μmole but less than 1500 μmole of carboxyl groups per gram of polysaccharide wherein said composition has reduced toxicity relative to composition with respect to lower levels of derivatization.

An embodiment of the invention provides a reduced polysaccharide iron oxide complex, such complex being stable at a temperature of at least approximately 100° C. In a preferred embodiment, such complex is stable at a temperature of approximately 121° C. In an even more preferred aspect of the reduced polysaccharide iron oxide complex, such complex is stable at a temperature of at least 121° C. for a time sufficient to sterilize the complex. In a further aspect of the embodiment, the iron oxide is superparamagnetic.

An embodiment of the invention provides a reduced derivatized polysaccharide iron oxide complex, such complex being stable at a temperature of at least approximately 100° C. In a preferred embodiment, such complex is stable at a temperature of approximately 121° C. In an even more preferred aspect of the reduced polysaccharide iron oxide complex, such complex is stable at a temperature of at least 121° C. for a time sufficient to sterilize the complex. In a further aspect of the embodiment, the iron oxide is superparamagnetic.

A preferred embodiment of the invention is a method of formulating for pharmacological use a reduced polysaccharide iron oxide complex having increased pH stability in comparison to the corresponding native dextran iron oxide, the method comprising: providing dextran; and reacting the dextran with a borohydride salt or hydrogen in the presence of an hydrogenation catalyst, reacting the reduced dextran with iron salts to provide a formulation having a stable pH.

A preferred embodiment of the invention is a method of formulating for pharmacological use a reduced derivatized polysaccharide iron oxide complex having increased pH stability in comparison to the corresponding native dextran iron oxide, the method comprising: providing dextran; and reacting the dextran with a borohydride salt or hydrogen in the presence of an hydrogenation catalyst, reacting the reduced dextran with iron salts to provide a formulation having a stable pH.

In another embodiment, the invention provides a method of formulating a reduced derivatized dextran composition for pharmacological use wherein the composition has decreased toxicity in comparison to native dextran; comprising: producing a reduced derivatized polysaccharide; and sterilizing the product by autoclaving. According to this method, the reduced polysaccharide is obtained by reacting the native polysaccharide with one of several reducing agents selected from the group consisting of a borohydride salt, or hydrogen in the presence of a hydrogenation catalyst. In a preferred aspect of the embodiment the polysaccharide is dextran. Producing the composition can include derivatizing a reduced polysaccharide by carboxyalkylation, for example, wherein the carboxyalkylation is a carboxymethylation. Further according to this method, the reduced polysaccharide can be a reduced dextran. The derivatized, reduced polysaccharide can be isolated as the sodium salt and does not contain an infrared absorption peak in the region of 1650–1800 cm^{-1}. In one aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than approximately 50° C. In another aspect of the method, producing the derivatized reduced polysaccharide is achieved at a temperature of less than approximately 40° C.

An embodiment of the invention provides an improved method of administering to a mammalian subject a reduced derivatized polysaccharide in a manner that the composition provides reduced toxicity, wherein the improvement comprises utilizing a reduced polysaccharide in formulation of the composition.

An embodiment of the invention provides a reduced polysaccharide, wherein the reduced polysaccharide is derivatized, for example, the reduced derivatized polysaccharide is a carboxyalkyl polysaccharide. The carboxyalkyl is selected from the group consisting of carboxymethyl, carboxyethyl and carboxypropyl. Further, the reduced polysaccharide can be a reduced dextran. A further aspect of this embodiment of the invention is that the level of derivatization of the reduced dextran is at least 750 micromolar of carboxyl groups per gram of polysaccharide wherein said composition has reduced toxicity relative to composition with lower levels of derivatization.

Another embodiment of the invention is a method of formulating a dextran composition for pharmacological use and having decreased toxicity in comparison to native dextran, the method comprising: providing dextran; and reacting the provided dextran with a borohydride salt or hydrogen in the presence of an hydrogenation catalyst followed by carboxymethylation, the reduced carboxymethylated dextran having decreased toxicity.

Another embodiment of the invention is an improved method of administering to a mammalian subject a polysaccharide composition of the type wherein the composition includes dextran in a manner that the composition provides reduced toxicity, wherein the improvement comprises utilizing reduced carboxymethylated dextran in lieu of dextran in the formulation. In another aspect, an embodiment of the invention is an improved method of administering to a mammalian subject a polysaccharide in a manner that the composition provides reduced toxicity, wherein the improvement comprises utilizing a reduced carboxymethylated polysaccharide in formulation of the composition.

An embodiment of the invention provides a method of use of reduced derivatized dextrans as blood expanders.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a Fourier transform infrared (FTIR) spectrographic analysis of carboxymethyl reduced dextran (CMRD) sodium salt obtained with Example 5.

FIG. 2 shows an FTIR spectrographic analysis of sodium salt CMRD coated ultrasmall superparamagnetic iron oxide (USPIO; see U.S. Pat. No. 5,055,288) obtained in Example 31.

FIG. 3 is a graph that shows the amount of carboxymethyl groups (micromoles) per gram of product, on the ordinate, as a function of the amount of bromoacetic acid mg/gram used in reactions with reduced dextran starting material, on the abscissa. The graph is plotted from the data of Table 2.

FIG. 4 shows pharmacokinetics of CMRD coated USPIO in the blood of three male rats following intravenous administration of 2.2 mg of iron per kg body weight. Samples (0.25 ml) of blood were collected at the times indicated on the abcissa, and relaxation times were measured on a Brucker Minispec spectrometer.

FIG. 5 shows the graph used to determine a half-life (67 minutes) of CMRD coated USPIO in rat blood. The data of FIG. 4 were used to generate the graph in FIG. 5. The half-life range of 61 to 75 minutes was within the 95% confidence level.

FIG. 6 shows MRIs of a rat, pre-administration (A) and post-administration (B) of contrast agents, anterior portion at top. CMRD coated USPIO (5 mg of iron per kg body weight) was administered into the femoral vein prior to taking the post administration contrast image. The figure illustrates enhanced visualization of the heart and surrounding arteries and veins caused by administration of CMRD coated USPIO. Imaging was performed using a General Electric 2 Tesla magnetic resonance imager.

FIG. 7 shows MRI images of a pig, pre-administration (A) and post-administration (B) of contrast agent, anterior portion at top. CMRD coated USPIO (Example 31; 4 mg of iron per kg body weight) was administered into the femoral vein prior to taking the post administration contrast image. The figure illustrates enhanced visualization of the heart and surrounding arteries and veins caused by administration of CMRD coated USPIO. Imaging was performed using a Siemans 1.5T Magnatom Vision magnetic resonance imager.

FIG. 8 shows MRI images of the anterior portion of a normal human subject, pre-administration (A) and post-administration (B) of contrast imaging agent. CMRD coated USPIO (4 mg of iron per kg body weight) was administered as a bolus into a vein in the arm prior to taking the post contrast image. Imaging was performed 15 to 30 minutes after administration of contrast agent. The image illustrates enhanced visualization of the heart and surrounding arteries and veins.

FIG. 9 shows the blood clearance kinetics in humans of imaging agent, CMRD coated USPIO (4 mg of iron per kg body weight), was administered as a bolus into a vein in the arm prior to taking blood samples. Samples were analyzed for 1/T2 relaxation to determine the blood concentration of the CMRD coated USPIO. The graph shows CMRD coated USPIO concentration (ordinate) as a function of time (abscissa).

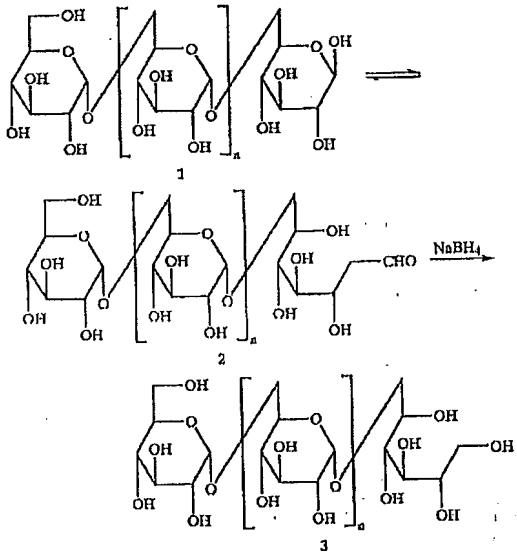
DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

Table 1 summarizes the characteristics of two classes of MRI contrast agents that have been previously described, and shows a comparison of their characteristics to those of an ideal contrast agent. Agents of the invention embody the ideal characteristics, as shown herein.

Surprisingly, the development and synthesis of preparations of ultrasmall superparamagnetic iron oxide (USPIOs) coated with polysaccharide reduced dextrans and derivatives of reduced dextrans, such as the agents with the desirable properties as shown herein, are derived from a change in the chemical nature of one constituent, dextran T10. This change involved reduction of the terminal aldehyde group to an alcohol of the polysaccharide used in its synthesis to an alcohol (Scheme 1). Scheme 1 illustrates the chemical change in a polysaccharide such as dextran upon treatment with sodium borohydride. The hemiacetal form of the polysaccharide (structure 1) is in equilibrium with the aldehyde form of the polysaccharide (structure 2). Structure 2 represents less than 0.01% of the equilibrium mixture (Brucker, G. (1974) *Organic Chemistry: Amino Acids, Peptides and Carbohydrates.*, Tankonykiado Press, Budapest, p. 991). Treatment of structure 2 with sodium borohydride results in its irreversible conversion to the linear polyol form of the polysaccharide (structure 3). The dynamic equilibrium between structures 1 and 2 allows complete

conversion, when treated with sodium borohydride, to the linear polyol (structure 3).

Scheme 1:



Dextran coated superparamagnetic iron oxide particles have particular interest as magnetic resonance imaging (MRI) contrast agents because of their ability to enhance images of the liver and lymph. Feridex I.V.® (Advanced Magnetics, Inc., Cambridge Mass.) is a dextran coated superparamagnetic iron oxide MRI contrast agent, and approved for use in humans. Combidex® (Advanced Magnetics, Inc.) is a dextran coated ultrasmall superparamagnetic iron oxide (USPIO) which has completed Phase III clinical trials for both liver imaging and Phase III trials for lymph imaging. Combidex® has a smaller mean diameter (20 nm) than Feridex I.V.® (60 nm), which gives it a different biodistribution in humans. Combidex® is made by addition of base to a solution of dextran, ferric chloride and ferrous chloride. The synthetic process comprises combining the ingredients, heating, and purifying by ultrafiltration. However, the yield of dextran added to the particles in the reaction is inefficient. Pharmaceutical grade dextran is the most expensive component of the Combidex® synthesis. A more efficient use of dextran in the synthesis of Combidex® is desirable to lower production costs.

Terminal sterilization (autoclaving) is a preferred method of sterilizing drugs for injection. However, many superparamagnetic iron oxide colloids that are used as MRI contrast agents are synthesized with polymer coatings and coverings that influence the biodistribution and elimination of these colloids. Upon exposure to the heat for the duration of the autoclaving process, the polymer coating can become dissociated from the iron oxide cores. The functional consequences of polymer dissociation from the iron oxide are physical changes in the material, such as clumping, biodistribution changes (changes in plasma half life), and changes in toxicity profile (potential increases in adverse events). For example, a substantial decrease in the pH of the solution can be detected following autoclaving of iron dextran particles, and the pH continues to fall upon further storage.

Several solutions to the problem of imparting resistance to heat stress have been described. Palmacci et al., U.S. Pat. No. 5,262,176, hereby incorporated herein by reference, used crosslinked dextran to stabilize the covering on the iron oxide particles prior to autoclaving. The crosslinking process uses noxious agents such as epichlorohydrin and epibromohydrin, which must be removed from the colloid after the crosslinking reaction.

Methods of preventing clumping of the colloid induced by heat stress that have no effect on coating dissociation have also been described. These methods generally include the use of excipients during the autoclaving process. Groman et al., U.S. Pat. No. 4,827,945, and Lowis et al., U.S. Pat. No. 5,055,288, both patents hereby incorporated herein by reference, use citrate to prevent clumping of the particles when the coating dissociates. Groman et al., U.S. Pat. No. 5,102,652, hereby incorporated herein by reference, uses low molecular weight carbohydrates such as mannitol to prevent clumping during autoclaving. These excipients increase the cost and complexity of manufacturing the product, yet do not solve the problem of dissociation of the polymer from the iron particle.

Josephson et al., U.S. Pat. No. 5,160,726, hereby incorporated herein by reference, avoids heat stress on the coating by using filter sterilization rather than heat to sterilize the colloid. Filter sterilization is expensive since both the sterilization process and container closure must be performed in a germ free environment. Additionally, filter sterilizing has a higher rate of failure than the process of autoclaving, which reflects the inability to obtain an environment for the filtration step that is entirely germ free.

Maruno et al., U.S. Pat. No. 5,204,457, describes a carboxymethyl-dextran coated particle with improved stability up to 80° C. for an extended period but does not teach use of terminal sterilization by autoclaving. Hasegawa et al. (Japan J. Appl. Phys., Part 1, 37(3A):1029–1032, 1998) describes carboxymethyl dextran coated iron particles with thermal stability at 80° C., but does not teach use of a carboxymethyl reduced dextran coated particle, nor of terminal sterilization by autoclaving.

Magnetic resonance imaging agents act by affecting the normal relaxation times, principally on the protons of water. There are two types of relaxation, one known as spin-spin or T1 relaxation, and the second known as spin-lattice or T2 relaxation. T1 relaxation generally results in a brightening of the image caused by an increase in signal. T1 processes are most useful in imaging of the vascular system. T2 relaxation generally results in a darkening of the image caused by a decrease in signal. T2 processes are most useful in imaging of organs such as the liver, spleen, or lymph nodes that contain lesions such as tumors. All contrast agents have both T1 and T2 properties; however, either T1 or T2 relaxation can characterize the dominant relaxation property of a particular contrast agent. Low molecular weight gadolinium based contrast agents are T1 agents, and have primary application in the imaging of vascular related medical problems such as stroke and aneurysms and the brain. Iron oxide based colloidal contrast agents are T2 agents, and have primary application in imaging tumors of the liver and lymph nodes (prostate and breast cancer). An agent possessing both T1 and T2 properties would be desirable. Using such an agent would (I) provide a single drug for all applications, and simplify the inventory of the pharmacy, (ii) simplify imaging in the MRI suite, and (iii) improve patient care by permitting simultaneous examination of multiple medical problems in a single patient during a single examination, rather than requiring use of either a T1 or a T2 contrast agent.

A dextran can elicit a sometimes fatal anaphylactic response when administered intravenously (i.v.) in man (Briseid, G. et al., *Acta Pharmcol. Et Toxicol.*, 1980, 47:119–126; Hedin, H. et al., *Int. Arch. Allergy and Immunol.*, 1997:113:358–359). Related adverse reactions have been observed also on administration of magnetic dextran coated iron oxide colloids. Non-magnetic dextran coated iron oxide colloids that have utility as hematinics, particularly as an adjunct to erythropoietin treatment for end stage renal dialysis patients, may have side effects.

Information regarding-anatomical features within the vascular system can be obtained using contrast agents in two ways. When the contrast agent is first administered as a bolus, it initially passes through the vascular tree as a relatively coherent mass. Coordinating the time of imaging of the desired anatomical feature to the time when the bolus passes through that feature can provide useful information. This technique of contrast agent use is called first pass imaging. At a later time, the bolus has been diluted by mixing, and attains an equilibrium concentration in the vascular system. Under certain circumstances, this equilibrium or steady state can offer useful information. Imaging can be performed at an early phase, within minutes after injection of the contrast agent ("first pass"), and at a later phase, from about ten minutes after injection of the contrast agent (equilibrium phase). Gadolinium agents are suited only for first pass imaging due to their ready diffusion from the vascular system into the interstitial spaces of the tissues. Previously described colloidal iron oxides are useful for the equilibrium due to their requirement for dilute administration over a prolonged time period. Colloidal iron oxides do not leak into the interstitial space but can remain in the vascular system for hours. An agent offering the opportunity to perform both first pass imaging and equilibrium imaging would be desirable.

During administration in a medical setting of a contrast agent for "first pass" imaging, the timing of imaging and passage of the "first pass" of the contrast agent may not coincide. If a useful image was not obtained, it becomes desirable to administer a second dose of contrast agent to obtain another "first pass" image. On other occasions radiologists find it useful to examine several volumes within the patient requiring a multiple dosing regimen of contrast agent in order to obtain "first pass" images at each of multiple sites of interest. With gadolinium contrast agents, this multiple administration "first pass" application is not possible because the gadolinium leaks out of the vascular space producing a fuzzy background around blood vessels of interest. Current iron oxide colloidal based contrast agents are not suitable as they are administered not as a bolus, but as a dilute solution over a long time, obviating "first pass" applications.

Diagnosis of tumor progression in cancer patients is important for characterizing the stage of the disease, and for assessing treatment. To minimize cost and discomfort to the patient, it is desirable in an MRI examination to administer a single dose of contrast agent that would allow assessment of multiple organ systems that might be affected by the disease. For instance, in primary breast cancer, it is desirable to assess tumor status in the breast and at multiple metastatic sites including the liver, spleen, bone marrow, and lymph nodes. Administration of gadolinium based contrast agents can not satisfy this requirement due to their short half life in the body, their leakage into the vascular system, and their inability to concentrate within organs of interest. Iron oxide colloid based contrast agents such as Combidex® can serve in this multiple capacity while Feridex I.V.®, another iron oxide colloid contrast agent, is limited to imaging the liver and the spleen.

Administration of a contrast agent in a small volume (less then 5 ml) is desirable, as small volume administration improves the resolution obtained from first pass imaging, and minimizes injection time and discomfort to the patient. Gadolinium based contrast agents are administered in volumes of about 30 ml due to constraints caused by the solubility and potency of these agents. Currently, iron oxide based contrast agents are administered as a dilute solution in a large volume (50–100 ml) over an extended period of time (30 minutes). These constraints arise from safety issues associated with the rapid and concentrated administration of iron oxide based agents. Bolus injection is desirable in that it allows first pass imaging and shortens contact time between the patient and health care provider. Further bolus injection allows the practitioner to administer the contrast agent while the subject is in the MRI apparatus during the examination, thereby optimizing efficient use of instrument imaging time. Gadolinium based agents can be administered as a bolus.

Gadolinium based contrast agents consist of a chelating molecule and the gadolinium cation. Gadolinium is a toxic element and must be excreted from the body to avoid toxicity. Colloidal iron oxides are not excreted from the body but are processed in the liver and other organs to metabolic iron, such as the iron in hemoglobin. Thus, compositions of the invention can serve as an iron supplement for patients suffering from anemia, and are especially useful for patients undergoing treatment with erythropoietin.

An embodiment of the invention provides a method for the synthesis of a colloid of an iron oxide associated with a water soluble polysaccharide coating in a manner that mitigates dissociation of the coating from the iron oxide when the material is subjected to heat stress.

As used herein and in the accompanying claims, "heat stress" is defined as heating the colloid to approximately 121° C. or higher for about 30 minutes at neutral pH, or other combinations of time, temperature, and pH that are well known in the art to autoclave (or terminally sterilize) an injectable drug.

A method that is an embodiment of the invention includes the steps of treating a polysaccharide with a reducing agent such a borohydride salt or with hydrogen in the presence of an appropriate hydrogenation catalyst such Pt or Pd to obtain the reduced polysaccharide, such that the terminal reducing sugar has been reduced to give an open chain polyhydric structure. The reduced polysaccharide may be an arabinogalactan, a starch, a cellulose, an hydroxyethyl starch (HES), an inulin or a dextran. Moreover, the polysaccharide may be further functionalized prior to particle formation. The method further comprises mixing the reduced polysaccharide with iron salts in an acidic solution selected from the group comprising ferric salts, ferrous salts, or a mixture of ferrous and ferric salts, cooling the solution, neutralizing the solution with a base, and recovering the coated iron oxide colloid.

In accordance with a further embodiment of the invention, the bases which may be employed are sodium hydroxide, sodium carbonate and more preferably, ammonium hydroxide, for the step of neutralizing the colloid. In a further embodiment of the invention, the polysaccharide derivative is reduced dextran and the iron salts may be ferrous and ferric salts, which produce a superparamagnetic iron oxide colloid with a water soluble coating that does not dissociate from the iron oxide core under heat stress during terminal sterilization.

In another embodiment of the invention, only ferric salts are employed, yielding a non-superparamagnetic particle.

In another embodiment, a coated colloid may be prepared by adding a polysaccharide to an iron oxide sol (a colloidal dispersion in a liquid), adjusting the pH to 6–8 and recovering the coated iron oxide colloid.

The term "colloid" as used in this specification and the accompanying claims shall include any macromolecule or particle having a size less than about 250 nm. The iron oxide polysaccharide colloids of the invention have substantially improved physical characteristics and manufacturability compared to previously described materials. Improved physical characteristics are evident in the ability of the colloid to withstand heat stress, as measured by subjecting the colloid to a temperature of 121° C. for 30 minutes. Colloid particles made according to the invention show less evidence of polysaccharide dissociation under stress, remaining colloidal, and exhibiting no appreciable change in size.

During manufacture, the process that is an embodiment of the invention typically uses one tenth or less the amount of polysaccharide compared to the amount required in previous preparations using non-reduced polysaccharide, resulting in substantial raw materials cost savings due to the improved efficiency of the process of the invention.

Variation in such factors as polysaccharide derivative concentration, base concentration and/or Fe(III)/Fe(II) concentration can produce colloids with different magnetic susceptibilities and sizes. Changing the Fe(III)/Fe(II) ratios changes the particle size and alters the magnetic susceptibility. Higher ratios (for example, 2.0 mol/mol) tend to decrease susceptibility, whereas lower ratios (for example, less than 1.5 mol/mol) tend to increase particle size.

The process may be adjusted to yield colloids with different biological properties by changing the type of polysaccharide, and further derivatizing the particle after synthesis.

The colloids that are an embodiment of the invention can be used as contrast agents for magnetic resonance imaging (MRI) or in other applications such as magnetic fractionation of cells, immunoassays, magnetically targeted drug delivery, and as therapeutic injectable iron supplements. These colloids are particularly suited to parenteral administration, because the final sterilization typically is autoclaving, a preferred method since it eliminates viability of all cellular life forms including bacterial spores, and viruses. Previous methods for making colloids required the addition of excipients such as citrate or low molecular weight polysaccharides as stabilizers during the autoclaving process (see U.S. Pat. Nos. 4,827,945 and 5,102,652), or avoided heat stress altogether by use of filter sterilization (see U.S. Pat. No. 5,150,726). Thus, the embodiments of the present invention comprising the colloid compositions, provide utilities as significantly improved MRI contrast agents, and hematinic agents that are iron supplements. The improvements provided in these agents over prior art are found in the following facts demonstrated in the examples herein: that the agents which are embodiments of the present invention are heat sterilizable by autoclaving, and are thus optimized for long-term storage at ambient temperatures; that these agents do not require the addition of excipients for maintenance of stability during the sterilization or storage processes; that the agents are non-toxic to mammals including humans at higher doses; that an effective dose of the agents used for imaging is a smaller amount of material than the agents described in the art; and that the pharmacokinetics following administration are such that iterated successive doses administered after a brief interval after administration of a first dose can be used to obtain additional images during a single clinical visit and use of the imaging apparatus.

In the case of dextran and derivatives thereof, the formulations prepared by this method are less immuno-responsive in mammals, as shown by data obtained using a rat model, and in clinical trials in human subjects. The dextran- and dextran derivative-coated iron particles enhanced imaging of the heart, lungs, kidneys, and other organs and systems in three mammalian species: rat, pig, and human. The dextran- and dextran derivative-coated iron particles can be used also as hematinic agents, to provide iron in a more efficiently absorbed format than is true of oral iron supplements, to groups of patients who are chronically iron-deprived, such as dialysis patients, cancer patients, gastroenteritis patients, and recipients of erythropoietin. The derivatized reduced dextrans can be used also as plasma extenders, which, unlike blood and blood fractions, do not have to be cross-matched immunologically, and unlike human serum albumin preparation, can be sterilized in a manner that destroys viruses, including strains of hepatitis, CMV, and HIV, spongiform encephalitis, and other infectious agents. The plasma extenders of the invention do not have to be refrigerated or stored away from light and heat, and are thus advantageous in emergency medical situations, such as treatment of shock due to loss of blood such as trauma, even in tropical climates.

Examples 1, 2 and 3 show the methods for making reduced dextrans of type T1, T5, and T10, respectively. Example 4 describes preparation of reduced pullulan.

Examples 5–9 describe the synthesis of carboxymethyl reduced dextran T10 with varying degrees of carboxymethylation, from native dextran T10. (Table 2).

Examples 10–15 describe the synthesis of carboxymethyl reduced dextran T10 with varying degrees of carboxymethylation, starting with reduced dextran T10 (Table 3).

Examples 16–18 describe the synthesis of carboxymethyl dextran T10, T40, and T70 from native dextran.

Examples 19–26 describe the preparation of reduced and native dextran coated iron oxides. The conditions of the reactions in these examples were chosen to yield USPIOs coated either with reduced or non-reduced polysaccharides. The reactions conditions for the native dextran iron oxide preparations were the same as for the reduced dextran preparations of the same molecular weights, to allow comparison of the effectiveness of the respective dextrans in coating particles. Mean volume diameter (MVD) and magnetic susceptibility of iron oxide preparations obtained using reduced in comparison to native polysaccharides (prepared in these examples) are summarized in Table 4.

Examples 27–29 describe a procedure for the preparation of USPIOs with native T1, T5, and T10 dextrans, to obtain iron oxide colloids having a particle diameter of less than 30 nm. A comparison of effects of native dextrans (Examples 27–29) and their respective reduced dextrans (Examples 19, 21, and 23) in the synthesis and properties of iron oxide colloids is shown in Table 5.

Examples 30–31 describe the preparation USPIOs coated with carboxymethyl native dextran T10 and carboxymethyl reduced dextran T10.

Examples 32–41 describe the preparation of USPIOs coated with carboxymethyl reduced dextran T10 preparations containing varying extents of carboxymethylation. The effect of extent of carboxymethylation of CMRDs on colloid

size of USPIOs is shown in Table 6. The effect of extent of carboxymethylation of CMRDs on solubility of ferric/ferrous chloride solutions is shown in Table 7.

Examples 42–48 describe the synthesis of iron oxide sols and their stabilization with native and reduced dextrans and CMRD. Example 49 describes preparation of CMRD coated non-magnetic iron oxide colloid using base precipitation of ferric chloride and CMRD.

Example 50 examines the effect of the process of sterilization by autoclaving of various preparations of USPIOs coated with reduced and native dextrans on the properties of these particles. The results are shown in Tables 8 and 9.

Example 51 reports the relaxation properties of various contrast agents comparing these properties for gadolinium based contrast agents and USPIOs prepared with native dextran and carboxymethyl reduced dextran T10 (Table 10).

In Examples 52–53, the presence of symtoms of toxicity to rats at doses in vast excess of reduced and non-reduced (native) dextran coated USPIOs was determined, with response to an anaphylactic type reaction. The extent of the anaphylatic type reaction is determined by volume of paw edemia. Similar studies were performed using native, reduced, and carboxymethylated reduced dextrans. The result are summarized Tables 11–14.

Example 54 and FIGS. 4 and 5 show the kinetics of clearance of a CMRD coated USPIO from rat circulation. The half-life of the agent is determined.

An enhanced MRI scan is shown in Example 55 and FIG. 6 following administration of CMRD coated USPIO, the scan showing images of the rat heart, aorta and other cardiac-associated arteries. Example 56 and FIG. 7 show a CMRD coated USPIO enhanced MRI scan of the anterior portion of a pig. Example 57 shows that injection of CMRD coated USPIOs into human subjects, as part of a clinical trial, produced no adverse effects. Example 57 describes the biodistribution (FIG. 8), imaging kinetics (FIG. 9 and Table 15), and absence of background in MRI usage of this material in humans. The data in this example show the ability of the practitioner of the invention to perform multiple administrations and obtain subsequent images within the real time of an office visit or visit to a MRI facility.

EXAMPLES

General Procedures for the Synthesis of Reduced Polysaccharides

Reduced polysaccharides were prepared by treatment with excess sodium borohydride and generally purified using five cycles of ultrafiltration. Distilled water is used throughout the examples. In the case of the polysaccharide pullulan, the reduction mixture was used without further purification. In all cases, the products showed less than 5% residual aldehyde content Residual aldehyde concentration was determined using a modified tetrazolium blue assay (Jue, C. K. et al., *J. Biochem. Biophys. Methods*, 1985, 11:109–15). Dextran concentration was determined by a phenol/sulfuric acid assay (Kitchen, R., *Proc. Sugar Process. Res. Conf*, 1983, 232–47). In cases where ultrafiltration was omitted, it was demonstrated that, except for the dextran T1, the residual borate salts did not affect particle formation. Examples 1 through 4 provide methods of synthesis of reduce polysaccharides T1, T5, and T10 dextrans, and pullulan, respectively. Retention times were determined using a Waters Ultrahydrogel 250 column, SN T52262A33, with 20 mM phosphate buffered saline, 0.4 ml/min flow rate.

Example 1

Reduced Dextran T1

Dextran T1 (10 g) was dissolved in 100 ml water at 25° C., 1.0 g of sodium borohydride was added, and the mixture was stirred for 12 h. The pH was brought to 5.0 using 6 M HCl, and 200 ml ethanol (anhydrous) was added. The precipitate was collected by centrifugation. The ethanol/water layer was decanted, and the residue was dissolved in 100 ml water. Addition of 200 ml of absolute ethanol was used to cause a second precipitation, and the ethanol/water was again decanted. The precipitated product was dissolved in water, and was lyophilized to produce a white solid, with a 60% yield. The observed HPLC retention times (min) were: for reduced dextran, 24.4; and for native dextran, 24.4.

Example 2

Reduced Dextran T5

Dextran T5 (4 g) was dissolved in 25 ml water at 25° C., 83 mg of sodium borohydride was added, and the mixture was stirred for 12 h. The pH was brought to 5.0 using 6 M HCl. The mixture was ultrafiltered against a 1 kDa molecular weight cut-off (MWCO) membrane filter. The product was lyophilized to produce a white solid, and a 70% yield was obtained. The observed HPLC retention times (min) were: for reduced dextran, 22.9; for native dextran, 21.9.

Example 3

Reduced Dextran T10

Dextran T10 (5,003 g) was dissolved in 26,011 g water. Sodium borohydride was added (52.5 g) and the mixture was stirred for 24 hours. The pH was adjusted to 7.1 using 6 N HCl. The product was purified by repeated ultrafiltration against a 3 kDa ultrafiltration membrane and lyophilized to produce a white solid. Yield: 3129 g. The observed HPLC retention times (min) were: for reduced dextran, 21.6; for native dextran, 21.1.

Example 4

Reduced Pullulan

Pullulan (90 mg) was dissolved in 0.8 ml water at 25° C., and 1 mg of sodium borohydride was added. The mixture was stirred for 12 h, and was used directly in the preparation of USPIO.

General Procedures for Synthesis of a Carboxymethyl Reduced Dextran Using Native Dextran T-10 as a Substrate

Examples 5–9 describe the synthesis of carboxymethyl reduced dextrans from native dextran. Two general methods of synthesis are presented, a low dextran concentration method (Example 5) in which the starting concentration of native dextran was 70 mg/g, and a high dextran concentration method (Examples 6–9), in which the starting concentration of native dextran was 240 mg/g.

Example 5

Carboxymethyl Reduced Dextran T10 Prepared by the Low Dextran Concentration Method

The following solutions were prepared and cooled to 5° C.: Solution A contained 4,200 g sodium hydroxide in 10.5 liters of water; and Solution B contained 2,310 g bromoacetic acid in 5,700 ml water. Solution C contained 3,000 g dextran T10 in 7,500 ml water, heated to 38° C.

Sodium hydroxide (600 g) was dissolved in 7.5 liters of water and was warmed to 38° C. Sodium borohydride (60 g) was added and the mixture was stirred for 2 min before adding Solution C, followed immediately by adding a sec-

ond 60 g portion of sodium borohydride. The mixture was stirred at 38° C. for 30 min, and then cooled to 15° C. Solution A was added, keeping the temperature of the solution below 25° C. Solution B was added, and the temperature of the solution was maintained below 25° C. The mixture was stirred for 2 hours at room temperature, and was neutralized to, pH 7.5 using 6M HCl cooled to 5° C., maintaining the solution temperature below 35° C. The mixture was filtered though a 0.2 μm filter, and diluted to 80 liters. The product was purified by repeated ultrafiltration through a 3 kDa MWCO ultrafiltration membrane, again filtered through a 0.2 μm filter and was lyophilized.

The recovered solid, 2,560 g of carboxymethyl reduced dextran T10 (sodium salt), showed a carboxyl content of approximately 1,265 micromoles carboxyl per gram of product, as determined by titration. The use of bromoacetic acid allowed the reaction to proceed at a lower temperature compared to use of chloroacetic acid, and produced a cleaner product as evidenced by its FTIR spectrum (FIG. 1). FIG. 1 shows no carbonyl absorption other than that of the carboxylate at 1600 cm^{-1}, unlike the FTIR of the product in U.S. Pat. No. 5,204,457 which was prepared with chloroacetic acid.

Example 6

Carboxymethyl Reduced Dextran CMRD T10 Prepared by the High Dextran Concentration Method

Sodium borohydride (6.4 g) and 0.5 g of a 50% solution weight/weight of sodium hydroxide in water were added to a solution of 25 g dextran in 50 g water. The mixture was stirred 4 hours at room temperature, 19.5 g of the 1:1 sodium hydroxide solution and 6.2 g bromoacetic acid were added, and the temperature was kept below 25° C. using an ice bath. The mixture was then stirred 16 hours at room temperature.

To purify the product, the pH of the mixture was adjusted to pH 6.2 using 6 M HCl, and 120 ml ethanol was added. A precipitate formed and was allowed to settle, and the supernatant was removed by decanting. The residue was dissolved in 60 ml water, and 200 mg sodium chloride was added, followed by 30 ml ethanol, and the carboxymethyl reduced dextran was allowed to settle out. The sequence of addition of water and sodium chloride followed by dissolution of the precipitate and ethanol precipitation, was repeated an additional two times. The residue was dissolved in 60 ml water, and 1 liter of ethanol was added. The carboxymethyl reduced dextran was again allowed to settle out, and the solid was collected on a medium frit glass filter. The white solid was dried 24 hours at 50° C. The yield was 27 g of product having 1108 micromoles carboxyl per gram as measured by titration (Table 2).

Example 7

Carboxymethyl Reduced Dextran T10 Prepared by the High Dextran Concentration Method

Sodium borohydride (0.4 g) and 0.5 g of 50% sodium hydroxide were added to a solution of 25 g dextran in 50 g water. The mixture was stirred 4 hours at room temperature, 20.0 g 50% of sodium hydroxide and 6.95 g of bromoacetic acid were added and temperature was kept below 25° C. using an ice bath while the mixture was stirred for 16 hours at room temperature. The product was purified as described in Example 6. The yield was 23.9 g of product having 1262 micromoles carboxyl per gram as measured by titration (Table 2).

Example 8

Carboxymethyl Reduced Dextran T10 Prepared by the High Dextran Concentration Method

Sodium borohydride (0.4 g) and 0.5 g of 50% sodium hydroxide were added to a solution of 25 g dextran in 50 g water. The mixture was stirred for 4 hours at room temperature, and 20.67 g of 50% sodium hydroxide and 7.65 g bromoacetic acid were added while the temperature was kept below 25° C. using an ice bath. The mixture was stirred for 16 hours at room temperature. The product was purified as described in Example 6. The yield was 24.5 g of product having 1404 micromoles carboxyl per gram as measured by titration (Table 2).

Example 9

Carboxymethyl Reduced Dextran CMRD T10 Prepared by the High Dextran Concentration Method

Sodium borohydride (0.4 g) and 0.5 g of 50% solution of sodium hydroxide were added to a solution of 25 g dextran in 50 g water. The mixture was stirred for 4 hours at room temperature, and 20.67 g of 50% sodium hydroxide and 7.65 g of bromoacetic acid were added while the temperature was kept below 25° C. using an ice bath. The mixture was stirred for 16 hours at room temperature, and the product was purified as described in Example 6. The yield was 23.4 g of product having 1528 micromoles carboxyl per gram of product as measured by titration (Table 2).

The relationship between amount of bromoacetic acid used in the synthesis and the resulting incorporation of micromoles of carboxyl groups into dextran was examined using the high dextran concentration method. The relationship was found to be linear (see Table 2 and FIG. 3). Reactant masses and carboxmethyl yields for Examples 6 through 9 are shown in Table 2.

TABLE 2

Conditions for CMRD synthesis extent and degree of carboxymethylation of the product.

Example	dextran mg/g	NaOH, mg/g	bromoacetic acid, mg/g	micromoles COOH per g product
6	246	96.0	61.0	1108
7	243	97.2	67.6	1262
8	240	99.2	73.4	1404
9	238	100.3	77.2	1528

Synthesis of Carboxymethyl Reduced Dextran Preparations Using Reduced Dextran T-10 by the Low Dextran High Base Method

Examples 10–14 describe the synthesis of carboxymethyl reduced dextrans with varying degrees of substitution starting with a low concentration of reduced dextran. In this method, the starting concentration of reduced dextran was 70 mg/g and the NaOH was at least about 107 mg/g. Table 3 shows that the extent of carboxymethyl substitution increased as the amount of bromoacetic acid used in the reaction increased.

Example 10

Carboxymethyl Reduced Dextran CMRD T10 Using the Low Dextran High Base Method

Reduced dextran T10 (15 g) was dissolved in 72 ml water, and 72 ml of 8M sodium hydroxide was added. The mixture

was brought to 25° C., and a solution of 1.15 g bromoacetic acid in 3 ml of water was added. The mixture was stirred at room temperature for 1 hour, and then added to a 75 ml volume of crushed ice. The pH of the solution was brought to pH 6.0 using 6M HCl. After repeated ultrafiltration against a 3 kDa ultrafiltration membrane, the product was lyophilized. The yield was 13.25 g of product. The recovered solid, carboxymethyl reduced dextran T10 (sodium salt), showed a carboxyl content of approximately 110 micromoles carboxyl per gram as determined by titration (Table 3).

Example 11

Carboxymethyl Reduced Dextran T10 Using the Low Dextran High Base Method

Reduced dextran T10 (150 g) was dissolved in 720 ml water, and 720 ml of 8M sodium hydroxide was added. The mixture was brought to 25° C., and a solution of 11.5 g bromoacetic acid in 140 ml water was added. The mixture was stirred at room temperature for 1 hour, added to a 750 ml volume of crushed ice, and the pH of the solution was brought to pH 6.0 with 6M HCl. After repeated ultrafiltration against a 3 kDa MWCO ultrafiltration membrane, the product was lyophilized. The yield was 126.21 g of recovered solid carboxymethyl reduced dextran T10 (sodium salt), having a carboxyl content of approximately 130 micromoles carboxyl per gram product as determined by titration (Table 3).

Example 12

Carboxymethyl Reduced Dextran CMRD T10 Using the Low Dextran High Base Method

Reduced dextran T10 (150 g) was dissolved in 720 ml water, and 720 ml of 8M sodium hydroxide was added. The mixture was brought to 25° C., a solution of 26.6 g bromoacetic acid in 140 ml water was added, and the mixture was stirred at room temperature for 1 hour and added to a 750 ml volume of crushed ice. The pH of the solution was brought to pH 6.0 with 6M HCl. After repeated ultrafiltration against a 3 kDa MWCO ultrafiltration membrane, the product was lyophilized. The yield was not determined. The recovered solid, carboxymethyl reduced dextran T10 (sodium salt), showed a carboxyl content of approximately 280 micromoles carboxyl per gram product as determined by titration (Table 3).

Example 13

Carboxymethyl Reduced Dextran CMRD T10 Using the Low Dextran High Base Method

Reduced dextran T10 (15 g) was dissolved in 72 ml of water, and 72 ml of 8M sodium hydroxide was added. The mixture was brought to 25° C., and a solution of 3.45 g of bromoacetic acid in 8 ml water was added. The mixture was stirred at room temperature for 1 hour, and then added to a 75 ml volume of crushed ice. The pH of the solution was brought to pH 6.0 with 6M HCl. After repeated ultrafiltrations against 3 kDa MWCO ultrafiltration membranes, the product was lyophilized. The yield was 9.4 g of recovered solid carboxymethyl reduced dextran T10 (sodium salt), having a carboxyl content of approximately 450 micromoles carboxyl per gram product as determined by titration (Table 3).

Example 14

Carboxymethyl Reduced Dextran CMRD T10 Using the Low Dextran High Base Method

Reduced dextran T10 (150 g) was dissolved in 720 ml of water, and 720 ml of 8M sodium hydroxide was added. The mixture was brought to 25° C., and a solution of 58.8 g of bromoacetic acid in 140 ml water was added. The mixture was stirred at room temperature for 1 hour, and was then added to a 750 ml volume of crushed ice. The pH of the solution was brought to pH 6.0 using 6M HCl. After repeated ultrafiltrations against a 3 kDa MWCO ultrafiltration membrane, the product was lyophilized. The yield was 127.88 g of the recovered solid carboxymethyl reduced dextran T10 (sodium salt), having a carboxyl content of approximately 580 micromoles carboxyl per gram product as determined by titration (Table 3).

Table 3 shows that the extent of carboxymethyl substitution observed was a function of the amount of bromoacetic acid used in the reaction. The data show that generally increasing the amount of bromoacetic acid in the reaction resulted in increasing levels of COOH in the product. The yield of carboxymethyl incorporation was also affected by conditions such as scale of the reaction, for example, as in Examples 13 and 14.

TABLE 3

Preparation of CMRDs with varying extents of carboxymethylation.

Example	dextran mg/g	NaOH mg/g	bromoacetic acid mg/g	micromoles COOH/g product
10	75	115.7	5.77	110
11	75	115.7	5.77	130
12	73	111.6	16.7	280
13	70	107.2	27.3	450
14	70	107.2	27.3	580

Example 15

Carboxymethyl Reduced Dextran T10 from a Commercial Source

Carboxymethyl reduced dextran was purchased from Amersham-Pharmacia. The solid showed a carboxyl content of approximately 1887 micromoles carboxyl per gram product as determined by titration.

Examples 16–18 describe synthesis of carboxymethyl dextran from native, non-reduced dextran T-10, T-40, and T-70, respectively.

Example 16

Carboxymethyl Dextran T10

The following solutions were prepared and cooled to 5° C.; Solution A: 105.2 g sodium hydroxide in 250 ml water; Solution B: 58.0 g bromoacetic acid in 142.5 ml water; and Solution C: 75.7 g dextran T10 in 187.5 ml water.

To Solution C and Solution A were added sodium hydroxide (14.4 g) dissolved in 187.5 ml water while maintaining the temperature of the solution below 25° C. Solution B was added, keeping the temperature below 25° C., and the resulting solution was stirred for 2 hours at room temperature, then was neutralized to pH 7.5 with 6M HCl (cooled to 5° C.) while maintaining the solution temperature below 35° C. The mixture was passed through a 0.2 μm pore size filter, and diluted to 2 liters. The product was purified by repeated ultrafiltration against a 3 kDa MWCO ultrafiltration membrane, 0.2 μm filtered again, and lyophilized. The yield was 53.17 g, and the recovered solid carboxymethyl dextran T10 (sodium salt) showed a carboxyl content of approximately 1220 micromoles carboxyl per gram product as determined by titration.

Example 17

Carboxymethyl Dextran T40

The following solutions were prepared and cooled to 5° C.: Solution A: 154 g sodium hydroxide in 480 ml water; Solution B: 77 g bromoacetic acid in 260 ml water; and Solution C: 100 g dextran T40 in 400 ml water.

Solution A was added to Solution C all at once. After 5 min, Solution B was added and the combined solution was stirred for 120 min while the temperature was maintained between 20° C. and 25° C. The mixture was neutralized with 6 M HCl, was 0.2 μm filtered, and diluted to 2 liters. The product was purified by repeated ultrafiltration against 3 kDa MWCO ultrafiltration membranes, was 0.2 μm filtered, and was lyophilized. The yield was 105.1 g of recovered solid carboxymethyl dextran T40 (sodium salt), which showed a carboxyl content of about 1390 micromoles carboxyl per gram product as determined by titration.

Example 18

Carboxymethyl Dextran T70

The following solutions were prepared and cooled to 5° C.: Solution A: 154 g sodium hydroxide in 480 ml water; Solution B: 77 g bromoacetic acid 260 ml water; and Solution C: 100 g dextran T70 in 400 ml water.

Solution A was added to Solution C all at once. After 5 min, Solution B was added, and the combined solution was stirred, maintaining the temperature between 20° C. and 25° C. using an ice bath. After 120 min, the solution was neutralized with 6 M HCl. The solution was 0.2 μm filtered, and diluted to 2 liters. The product was purified by repeated ultrafiltration against 3 kDa MWCO ultrafiltration membranes, was 0.2 μm filtered again and was lyophilized. The yield was 106.9 g of recovered solid carboxymethyl dextran T70 (sodium salt), having a carboxyl content of about 1380 micromoles carboxyl per gram product as determined by titration.

General Procedure for the Preparation of Superparamagnetic Colloids for Comparison of the Properties of USPIO Preparations Coated with Either of Reduced or Non-reduced Polysaccharides

Examples 19–26 were conducted to compare polysaccharide coated iron oxide products obtained from pairs of native and reduced polysaccharides of identical molecular weights. Identical procedures were utilized for the preparation of USPIO colloids for each pair of native and reduced polysaccharide of identical molecular weight. In particular, the same polysaccharide to iron ratio and iron concentration was used for each molecular weight pair. The polysaccharide to iron ratio and iron concentration utilized for each native and reduced polysaccharide pair were chosen to yield a 0.2 μm filterable USPIO with a diameter of less than 30 nm and a magnetic susceptibility of greater than $20{,}000\times10^{-6}$ cgs with the reduced polysaccharide.

The general procedure involved addition of excess ammonium hydroxide to a solution of iron salts (Fe^{+3}/Fe^{+2}) and polysaccharide, followed by heating, and performing six cycles of ultrafiltration against water using a 100 kDa MWCO membrane filter. After ultrafiltration, the USPIO preparations formed with reduced polysaccharide were filtered through a 0.2 μm filter and stored at 4° C.

It was observed that for iron oxides prepared with a native polysaccharide, only the native dextran T10 coated iron oxide was filterable through a 0.2 μm filter. The size and magnetic susceptibility, except for those samples containing particulate materials, were then measured. Particle sizes were determined by measurement of dynamic light scattering in a Microtrac® UPA instrument (Honeywell IAC Microtrac, Fort Washington, Pa.) and are reported as the mean volume diameter (MVD). Magnetic susceptibility was determined with a Mathey Johnson magnetic susceptibility balance. Iron concentrations were determined with a bipyridyl assay (Kumar K., *J. Liq. Chromatogr. Relat. Technol.*, 1997, 20, 3351–3364).

Example 19

Preparation of Reduced Dextran T1 Coated USPIO

Reduced dextran T1 (1.7 g) was dissolved in 20 ml water, and a solution of 3 g of ferric chloride hexahydrate and 1.5 g of ferrous chloride tetrahydrate in 32 g water was added. The mixture was purged with nitrogen for 30 min, cooled to 5° C., and 12.7 g of 28% ammonium hydroxide was added with stirring during a 2 min period. The mixture was heated to 60° C., maintained at this temperature for 40 min, then incubated at 80° C. for 2 h. The product was subjected to six cycles of ultrafiltration against water using a 100 kDa MWCO membrane filter. After ultrafiltration, the product was filtered through a 0.2 μm filter and stored at 4° C. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 18 nm; the magnetic susceptibility was $13{,}323\times10^{-6}$ cgs/g Fe.

Example 20

Preparation of Native Dextran T1 Coated Iron Oxide

Native dextran T1 iron oxide was prepared by the method described above for the reduced dextran in Example 19 except that native dextran T1 was used instead of reduced dextran T1. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 2764 nm; the magnetic susceptibility was $1{,}953\times10^{-6}$ cgs/g Fe.

Example 21

Preparation of Reduced Dextran T5 Coated USPIO

Reduced dextran T5 (0.45 g) was dissolved in 13 ml water, and a solution of 0.5 g of ferric chloride hexahydrate and 0.25 g of ferrous chloride tetrahydrate in 4.5 g water was added. The mixture was purged with nitrogen for 30 min, cooled to 5° C., and 1.42 g of 28% ammonium hydroxide was added with stirring during a 2 min period. The mixture was heated at 80° C. for 2 h, and was purified as described in Example 19. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 16 nm; the magnetic susceptibility was $33{,}943\times10^{-6}$ cgs/g Fe.

Example 22

Preparation of Native Dextran T5 Coated Iron Oxide

Native dextran T5 iron oxide was prepared by the method described above for the reduced dextran in Example 21 except that native dextran T5 was used instead of reduced dextran T5. The product was observed to have the following

properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 1,916 nm.

Example 23

Preparation of Reduced Dextran T10 Coated USPIO

Reduced dextran T10 (2.7 g) was dissolved in 70 ml water, and a solution of 2.0 g ferric chloride hexahydrate and 1.0 g ferrous chloride tetrahydrate in 27 g water was added. The mixture was purged with nitrogen for 30 min, cooled to 5° C., and 8.5 g of 28% ammonium hydroxide was added with stirring during a 2 min period. The mixture was heated at 80° C. for 2 h and purified as described in Example 19. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 12 nm; the magnetic susceptibility was $31,743\times10^{-6}$ cgs/g Fe.

Example 24

Preparation of Native Dextran T10 Coated Iron Oxide

Native dextran T10 iron oxide was prepared by the method described above for the reduced dextran in Example 23 except that native dextran T10 was used instead of reduced dextran T10. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 757 nm; the magnetic susceptibility was $31,252\times10^{-6}$ cgs/g Fe.

Example 25

Preparation of Reduced Pullulan Coated USPIO

Reduced pullulan (0.045 g) was dissolved in 0.4 ml water, and a solution of 0.106 g ferric chloride hexahydrate and 0.05 g ferrous chloride tetrahydrate in 1.3 g water was added. The mixture was purged with nitrogen for 30 min, cooled to 5° C., and 0.044 g of 28% ammonium hydroxide was added with stirring during a 2 min period. The mixture was heated at 80° C. for 0.67 h and purified as described in Example 19. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 20 nm; the magnetic susceptibility was $27,066\times10^{-6}$ cgs/g Fe.

Example 26

Preparation of Native Pullulan Coated Iron Oxide

Native pullulan iron oxide was prepared by the method described above for the reduced pullulan in Example 25 except that native pullulan was used instead of reduced pullulan. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 1,184 nm.

Properties of Iron Oxide Preparations Obtained Using Reduced in Comparison to Native Polysaccharides (Comparison of Data Obtained from Examples 19–26)

In general for MRI contrast agents, an iron oxide contrast agent particle of small size is preferred, for example, a particle having a diameter in the range of 10 to 50 nm. Further, an iron oxide of greater magnetic susceptibility, and of greater homogeneity is preferred.

It is observed from the data of Examples 19–26 that the presence of a reduced terminal sugar of a polysaccharide (reduced polysaccharide) used to coat an iron oxide had an unexpected and substantial effect on the diameter of particles of each of the resulting colloids, compared to similarly produced iron oxides made using native non-reduced polysaccharide. Table 4 shows the size of particles formed for each pair of native and reduced polysaccharides, as indicated by the mean volume diameters (MVD). The concentration of reduced and native polysaccharides were kept constant within each molecular weight group. Concentrations were selected to optimize the synthesis of USPIO with reduced polysaccharide. For all polysaccharides, use of the native non-reduced polysaccharide consistently produced a larger particle than did use of the reduced dextran, so that the reduced polysaccharide consistently gave the preferred smaller particle.

Further, for each pair of polysaccharides of a given molecular weight that was synthesized and tested, the USPIO preparation coated with reduced polysaccharides demonstrated a higher magnetic susceptibility value than the corresponding iron oxide preparation synthesized with native polysaccharide, except for colloids obtained with dextran T10 for which magnetic susceptibilities of reduced and native coatings were equivalent.

These data indicate that use of a reduced polysaccharide in preparation of coated USPIO colloids yields preferred particles of small size, without loss of magnetic susceptibility. The data demonstrate the surprising effect that reduction of the aldehyde of a polysaccharide has upon the synthesis of a polysaccharide-coated USPIO.

TABLE 4

Comparison of properties of iron oxides made with native or reduced polysaccharides under conditions that form a USPIO with reduced polysaccharides.

Example	polysaccharide	ratio of polysaccharide per Fe, g/g	MVD nm		MS[a]	
			reduced	native	reduced	native
19, 20	dextran T1	1.6	18	2,764	13,323	1,953
21, 22	dextran T5	2.9	16	1,916	33,943	[b]
23, 24	dextran T10	4.6	21	757	31,743	31,252
25, 26	pullulan	3.9	20	1,184	27,066	[b]

[a]Magnetic susceptibility ($\times 10^{-6}$ cgs/g Fe)

[b]The sample was particulate, could not be filtered through a 0.2 μm filter, and magnetic susceptibility was not determined.

Properties of Iron Oxides Prepared with Native Non-reduced T1, T5 and T10 Dextrans of Mean Volume Diameter Less than 30 nm

Examples 27 through 29 show the preparation of iron oxides obtained from native dextran T1, T5, and T10. Colloids were prepared using non-reduced (native) dextrans as described for reduced dextrans (Examples 19, 21, and 23), except that the preparation of these native non-reduced dextran particles required about 10- to 34-fold more dextran than their corresponding reduced dextran counterpart to produce iron oxides of corresponding size. The requirement for increased dextran usage is shown by comparing the dextran to iron ratio of the products for corresponding molecular weight pairs of iron oxides shown (Table 5).

The data show that the magnetic properties, and the efficiency of dextran use during synthesis, of iron oxide particles prepared with each of native dextrans T1, T5, and T10 were inferior compared with corresponding properties of particles prepared with each counterpart reduced dextran.

Examples 27

Preparation of Iron Oxide Coated with Native T1 Dextran

A mixture of 0.42 g ferric chloride hexahydrate, 0.21 g ferrous chloride tetrahydrate, and 7.27 g water was filtered through a 0.2 μm filter. A 1.0 g portion of this mixture was added to 10 ml of an aqueous solution of 0.1 g dextran T1/g water. The mixture was purged with nitrogen before adding 0.22 ml of 28% ammonium hydroxide solution. The mixture was heated at 80° C. for 1 hour, cooled to room temperature and filtered through a 0.2 μm filter. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 27 nm; the magnetic susceptibility was 2325×10^{-6} cgs/g Fe.

Examples 28

Preparation of Iron Oxide Coated with Native T5 Dextran

Dextran T5 (0.8 g) was dissolved in 9 ml water, and added to 0.63 ml of a 0.2 μm filtered solution of 51.8 mg ferric chloride hexahydrate and 25.9 mg ferrous chloride tetrahydrate in 9.2 ml water. The mixture was purged with nitrogen before adding 1.4 ml 28% ammonium hydroxide solution. The mixture was heated at 80° C. for 1 hour, cooled to room temperature, and filtered through a 0.2 μm filter. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 20 nm; the magnetic susceptibility was 1285×10^{-6} cgs/g Fe.

Examples 29

Preparation of Iron Oxide Coated with Native T10 Dextran

Dextran T10 (9420 g) was dissolved in 14915 g water. A 14915 g portion of this mixture was filtered through a 0.2 μm filter, and added to the reaction vessel. Ferric chloride hexahydrate (891 g) was dissolved in 713 g water. A 1129 g portion was 0.2 μm filtered and added to the reaction vessel containing the dextran. The mixture was cooled to 5° C. with stirring overnight while bubbling nitrogen through the mixture. Before the last 30 min. of the nitrogen purge, a 580 g portion of a 0.2 μm filtered solution of 359 g ferrous chloride tetrahydrate in 477 g water was added. To this mixture was added 786 g of 28% ammonium hydroxide solution, cooled to 5° C. The mixture was heated to 80° C., incubated at 80° C. for 2 hours, and then poured into 80 liters of water heated to 80° C. The mixture was allowed to cool overnight, 0.2 μm filtered, and purified by ultrafiltration using a 100 kDa ultrafiltration membrane. The product was 0.2 μm filtered. The product was observed to have the following properties: the mean volume diameter (determined by use of a Microtrac Particle Size Analyzer) was 21 nm; the magnetic susceptibility was $32,712\times10^{-6}$ cgs/g Fe.

TABLE 5

Magnetic susceptibility and particle size properties of polysaccharide coated iron oxides: a comparison of native dextrans (Examples 27–29) with respective reduced dextrans (Examples 19, 21, and 23) under conditions to give particles of less than 30 nm MVD with maximum magnetic susceptibility

Example	dextran type	dextran/Fe (g/g)[b]	MVD (nm)	MS[a]
	iron oxides prepared with native dextran			
27	dextran T1	55	27	2,325
28	dextran T5	44	20	1,285
29	dextran T10	44	21	32,712
	iron oxides prepared with reduced dextran			
19	dextran T1	1.6	18	33,323
21	dextran T5	2.9	16	33,943
23	dextran T10	4.6	12	31,743

[a]Magnetic susceptibility ($\times10^{-6}$ cgs/g Fe)
[b]The polysaccharide/Fe ratio was varied for each dextran in order to obtain a USPIO with a MVD of less than or equal to 30 nm.

Preparation USPIOs Coated with Carboxymethyl Native Dextran T10 and Carboxymethyl Reduced Dextran T10 Containing Varying Degrees of Carboxymethylation

Examples 30 and 31 describe preparation of USPIO coated with carboxymethyl native and reduced dextran T10, respectively. Examples 32–36 describe the synthesis of USPIO compositions coated with carboxymethyl reduced dextran T10 preparations, containing varying degrees of carboxymethylation. Examples 37–41 describe the solubility of preparations containing ferric chloride and carboxymethyl reduced dextran T10 containing varying degrees of carboxymethylation.

Example 30

Preparation of USPIO Coated with Carboxymethyl Dextran T10

Carboxymethyl dextran T10 (60 g, prepared by the method Example 16) was dissolved in 532 g water. A solution of 14.7 g ferric chloride hexahydrate, 7.2 g ferrous chloride tetrahydrate, and 100 ml water, was filtered through a 0.2 μm, and added. The mixture was cooled to 10° C., purged with nitrogen, and 52.2 ml of 28% ammonium hydroxide solution was added with stirring. The mixture was heated to 75° C., maintained at 75° C. for 30 min, diluted with 2.5 liter water, and filtered through a 0.2 μm filter. The product was purified by repeated ultrafiltration against a 100 kDa MWCO membrane, concentrated to 20 mg Fe/ml, and again filtered through a 0.2 μm filter. The product was observed to have the following properties: MVD (determined by use of a Microtrac Particle Size Analyzer) was 19 nm; the magnetic susceptibility was $27,835\times10^{-6}$ cgs/g Fe; and the carboxyl content was 1,220 micromoles per gram of the CMRD. To determine stability in response

to autoclaving, a sample of the product was placed in a sealed 5 ml glass vial, and heated to 121° C. for 30 min (see Table 9).

Example 31

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10

Reduced carboxymethyl dextran T10 (40 g prepared in Example 5) was dissolved in 1,038 ml water and was filtered through a 0.2 μm pore size filter. A 0.2 μm filtered solution of 30 g ferric chloride hexahydrate and 15 g of ferrous chloride tetrahydrate in 374 ml of water was added to the dextran, with a 31 ml water wash. The solution was cooled to 10° C., and 114 g of 28% ammonium hydroxide was added. The colloidal mixture was heated to 78° C. and maintained at that temperature for one hour. The solution was then diluted to 3 liter with water, cooled to 10° C., and ultrafiltered 6 times with a YM-100 filter membrane (100 kDa MWCO). A final concentration of 21.1 mg Fe/g was obtained. The product was observed to have the following properties: the mean volume diameter (Microtrac Particle Size Analyzer) was 21 nm; the magnetic susceptibility was $32{,}732\times10^{-6}$ cgs/g Fe; and the carboxyl content was 1,265 micromoles per gram of the CMRD. The content of the particle was determined to be about 50% Fe and 50% dextran. To determine stability in response to autoclaving, a sample of the product was placed in a sealed 5 ml glass vial, and heated to 121° C. for 30 min (see Table 9).

Example 32

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10 Having 110 Micromoles Carboxyl per Gram

Carboxymethyl reduced dextran T10 (4 g, prepared in Example 10) was dissolved in 85 ml water. To this was added a 0.2 μm filtered mixture of 2.99 g ferric chloride hexahydrate, 1.49 g ferrous chloride tetrahydrate, and 37.3 ml water. The mixture was cooled to 10° C., purged with nitrogen, 11.4 g of 28% ammonium hydroxide solution was added with stirring the mixture was heated to 90° C., maintained at 78° C. for 60 minutes, and then maintained at 78° C. while bubbling air through the mixture. The mixture was diluted with 1.5 liters of water, and was filtered through a 0.2 μm filter. The product was purified by repeated ultrafiltration against a 100 kDa MWCO membrane and again filtered through a 0.2 μm filter.

Example 33

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10 Having 130 Micromoles Carboxyl per Gram

Carboxymethyl reduced dextran T10 (40 g, prepared in Example 11) was dissolved in 850 ml water. To this was added a 0.2 μm filtered mixture of 29.9 g ferric chloride hexahydrate, 14.9 g ferrous chloride tetrahydrate, and 373 ml water. The mixture was cooled to 10° C., purged with nitrogen, 114 ml of 28% ammonium hydroxide solution was added with stirring, the mixture was heated to 90° C., maintained at 78° C. for 60 min, and then maintained at 78° C. while bubbling air through the mixture. The mixture was diluted with 1.5 liters of water, and filtered through a 0.2 μm filter. The product was purified by repeated ultrafiltration against a 100 kDa MWCO membrane, concentrated to 20 mg Fe/ml, and again filtered through a 0.2 μm filter.

Example 34

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10 Having 280 Micromoles Carboxyl per Gram

Carboxymethyl reduced dextran T10 (4 g, prepared in Example 12) was dissolved in 85 ml water. To this was added a 0.2 μm filtered mixture of 2.99 g ferric chloride hexahydrate, 1.49 g ferrous chloride tetrahydrate, and 37.3 ml water. The mixture was cooled to 10° C., and purged with nitrogen. To the mixture was added with stirring 11.4 g of 28% ammonium hydroxide solution, the mixture was heated to 90° C., maintained at 78° C. for 60 min, and then maintained at 78° C. while air was bubbled through the mixture. The mixture was diluted with 1.5 liters of water, and filtered through a 0.2 μm filter. The product was purified by repeated ultrafiltration against a 100 kDa MWCO membrane, followed by filtration through a 0.2 μm filter.

Example 35

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10 Having 450 Micromoles Carboxyl per Gram

Carboxymethyl reduced dextran T10 (4 g, prepared in Example 13) was dissolved in 85 ml water. To this was added a 0.2 μm filtered solution of 2.99 g ferric chloride hexahydrate, 1.49 g ferrous chloride tetrahydrate, and 37.3 ml water. The mixture was cooled to 10° C., and purged with nitrogen before adding 11.4 g of 28% ammonium hydroxide solution with stirring. The mixture was heated to 90° C., maintained at 78° C. for 60 min, and then maintained at 78° C. while air was bubbled through the mixture. The mixture was diluted with 1.5 liters of water, filtered through a 0.2 μm filter, and was purified by repeated ultrafiltration against a 100 kDa MWCO membrane followed by filtration through a 0.2 μm filter.

Example 36

Preparation of USPIO Coated with Carboxymethyl Reduced Dextran T10 Having 580 Micromoles Carboxyl per Gram

Carboxymethyl reduced dextran T10 (40 g, prepared in Example 14) was dissolved in 85 ml water. To this was added a 0.2 μm filtered solution of 29.9 g ferric chloride hexahydrate, 14.9 g ferrous chloride tetrahydrate, and 373 ml water. The mixture was cooled to 10° C., purged with nitrogen, 11.4 g of 28% ammonium hydroxide solution with stirring. The mixture was heated to 90° C., maintained at 78° C. for 60 min, then maintained at 78° C. while bubbling air through the mixture. The mixture was diluted with 1.5 liters of water and filtered through a 0.2 μm filter, and was purified by repeated ultra-filtration against a 100 kDa MWCO membrane followed by filtration through a 0.2 μm filter.

The effect of degree of carboxymethylation of the CMRD coated USPIOs on colloid size was compared. Examples 31–36, Table 6. The MVD values of the resulting colloids were reasonably uniform between CMRD preparations con

taining 110 to 1265 micromoles of carboxyl per gram of product.

TABLE 6

Particle sizes of USPIO colloids prepared with dextran T10 CMRDs having varying degrees of carboxymethylation.

Example #	micromoles COOH/ g dextran	mean volume diameter, nm
32	110	12
33	130	15
34	280	18
35	450	16
36	580	20
31	1265	21

Example 37

Mixing of Carboxymethyl Reduced Dextran T10 Having 1,108 Micromoles Carboxyl per Gram with Ferric Chloride Solution

As a step in particle synthesis, ferric chloride (0.3 g) was dissolved in 15 ml water and was filtered through a 0.2 μm pore size filter. Carboxymethyl reduced dextran (prepared in Example 6) was added, the mixture was shaken, and was cooled to 10° C. No precipitate was observed.

Example 38

Mixing of Carboxymethyl Reduced Dextran T10 Having 1,262 Micromoles Carboxyl per Gram with Ferric Chloride Solution

Ferric chloride (0.3 g) was dissolved in 15 ml water and was filtered through a 0.2 μm pore size filter. Carboxymethyl reduced dextran (prepared in Example 7) was added, the mixture was shaken, and was cooled to 10° C. No precipitate was observed.

Example 39

Mixing of Carboxymethyl Reduced Dextran T10 Having 1,404 Micromoles Carboxyl per Gram with Ferric Chloride Solution

Ferric chloride (0.3 g) was dissolved in 15 ml water and was filtered through a 0.2 μm pore size filter. Carboxymethyl reduced dextran (prepared in Example 8) was added, the mixture was shaken, and was cooled to 10° C. No precipitate was observed.

Example 40

Mixing of Carboxymethyl Reduced Dextran T10 Having 1,528 Micromoles Carboxyl per Gram with Ferric Chloride Solution

Ferric chloride (0.3 g) was dissolved in 15 ml water and was filtered through a 0.2 μm pore size filter. Carboxymethyl reduced, dextran (prepared in Example 9) was added, the mixture was shaken, and was cooled to 5° C. An orange white precipitate was observed.

Example 41

Mixing of Carboxymethyl Reduced Dextran T10 Having 1,887 Micromoles Carboxyl per Gram with Ferric Chloride

Ferric chloride hexahydrate (30.3 g) and ferrous chloride (14.8 g) were dissolved in 402.9 ml water and filtered through a 0.2 μm pore size filter. Carboxymethyl reduced dextran T10 (40.3 g in 1,033 ml, prepared in Example 15) was added, the mixture was shaken, and was cooled to 5° C. An orange white precipitate was observed.

The effect of varying the degree of carboxymethylation of CMRDs on the first step of the CMRD-USPIO synthesis, i.e., combining the aqueous mixtures of CMRD with the iron chloride solutions, was analyzed. The various CMRD preparations were mixed with iron salts at a fixed iron concentration, the CMRD preparations differing only in degree of carboxymethylation as described in Examples 37–41. From 1,108 to 1,404 micromoles carboxyl per gram dextran, the CMRD formed a homogeneous mixture in the presence of ferric chloride (Table 7).

TABLE 7

Precipitation of CMRDs having varying levels of carboxyl groups after addition of iron salts from mixtures of CMRD (25 mg/g solution) and ferric chloride (19 mg/g solution).

Example #	micromoles COOH/g dextran	precipitate
37	1,108	no
38	1,262	no
31	1,265	no
39	1,404	no
40	1,528	yes, at 5° C.
41	1,887	yes, at 25° C.

At greater than 1,404 micromoles carboxyl per gram dextran, addition of ferric chloride under the conditions and concentrations of the USPIO synthesis to the CMRD solution produced an orange white precipitate. Even at higher temperatures, where many compounds can be soluble, the precipitates persisted. The data in Table 7 shows that there is an upper level in modification of CMRD that can be used in the preferred method of CMRD-USPIO synthesis.

Example 42

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Preparation of a Magnetic Sol

To prepare a magnetic sol, 60 g of 28% of ammonium hydroxide at 25° C. was added to a solution having 30.0 g ferric chloride hexahydrate and 15.1 g ferrous chloride tetrahydrate in 321 g of water. After 5 minutes of mixing, sufficient concentrated HCl was added to obtain a pH of 1.6. The sol was ultrafiltered with a 100 kDa MWCO membrane filter to achieve a pH of 3.25, using water as diluent. The magnetic sol was passed through a filter of pore size 0.2 μm, then concentrated to 50 mg Fe/g, and stored at 5° C. The yield of iron was 55%, and the product was observed to have an MVD of 16 nm.

Example 43

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Preparation of a Non-magnetic Sol

To a solution of 2.9 g of ferric chloride hexahydrate in 30 ml of water was added 10 ml of 10 M NaOH. The mixture was stirred for 5 min, diluted to 200 ml with water, and the product was collected by filtration. The residue was again mixed with water and filtered. The residue was added to 40 ml water and the pH was adjusted to 2.0. The product was observed to have an MVD of 10 nm.

Example 44

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Coating of a Magnetic Sol with Reduced Dextran T10

Reduced dextran T10 (60 mg; Example 3) was dissolved in 1.74 ml water and combined with 0.24 ml of magnetic sol (13 mg Fe) prepared according to Example 42. The mixture was incubated for 15 min, and the pH was adjusted to 7.4 with sodium hydroxide. The particle size (MVD) was determined to be 85 nm.

Example 45

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Coating of a Magnetic Sol with Native Dextran T10

Native dextran T10 (60.8 mg) was dissolved in 1.74 ml water, and combined with 0.24 ml of magnetic sol (13 mg Fe) prepared according to Example 42. The mixture was incubated for 15 min and the pH was adjusted to 7.4 with sodium hydroxide. The particle size (MVD) was determined to be 1,973 nm.

Example 46

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Coating of a Magnetic Sol with CMRD T10

75 mg of CMRD T10 (Example 5) dissolved in 1.34 ml water was added to 0.66 ml of magnetic sol (33 mg Fe) prepared according to Example 42. The mixture was incubated for 15 min at 37° C., and the pH was adjusted to 7.95 (plus or minus 0.4) with sodium hydroxide. The mixture was concentrated using a 300 kDa ultrafiltration filter. The product was observed to have an MVD of 41 nm.

Example 47

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Adjusting the pH of the Magnetic Sol to 7.4

A magnetic sol as prepared in Example 42 was adjusted to a pH of 7.4. A precipitate was observed.

Example 48

Synthesis of Iron Oxide Sols and Their Stabilization with Native and Reduced Dextrans and CMRD: Coating of a Non-magnetic Sol with CMRD T10

A non-magnetic sol prepared according to Example 43 (35 ml) was added dropwise to 35 ml of a 50 mg/g aqueous solution of CMRD T10 prepared according to Example 5. The pH was adjusted to 7.0 with 1 N NaOH, the solution was heated to boiling, cooled to room temperature, and was centrifuged at 6,000 rpm for 20 min. The supernatant was passed through a filter having a 0.2 μm pore size, and autoclaved at 121° C. for 30 min. The product was observed to have a MVD of 86 nm.

Examples 42–48 show that in the absence of a dextran, or in the presence of a native dextran, a gross iron precipitate forms. Only reduced dextran and CMRD yielded a magnetic sol as a stable colloid.

Example 49

Preparation of CMRD Coated Non-magnetic Iron Oxide Colloid Using Base Co-precipitation of Ferric Chloride and CMRD

Carboxymethyl reduced dextran T10 (19.2 g) (Example 5) was dissolved in 300 g water, was filtered through a 0.2 μm filter, and an additional 160.8 g of water was added. This solution was added to 120 ml of 0.2 μm filtered aqueous 0.3 M ferric chloride hexahydrate. To this mixture was added 32 ml of aqueous 6N sodium hydroxide. The mixture was heated to 100° C. for 3 hours, cooled to room temperature, and ultrafiltered to a final volume of 50 ml. The product was observed to have an MVD of 30 nm. A portion of this material was placed in a bottle under nitrogen for 30 min at 121° C. The autoclaved product had an MVD of 69 nm.

Example 50

Effect of Autoclaving on Reduced and Native Dextran Colloids: Stability to Autoclaving of USPIOs Coated with Native Dextran and Reduced Dextran and CMRD

Colloid preparations, each at a concentration of 20 mg Fe/g, were autoclaved for 30 min at 121° C. Following autoclaving, measurements were made of bound dextran which was calculated as the difference between total and free dextran, using a phenol/sulfuric acid assay. Free dextran was separated from the colloid by ultrafiltration. Table 8 shows that colloid preparations having USPIOs coated with a reduced dextran have greater stability than USPIOs coated with a native dextran. The reduced dextran coated USPIO maintained its small size following autoclaving, as the MVD of the post autoclaved material was increased only 1.3-fold compared to the MVD of the pre autoclaved material. In contrast, USPIO coated with native dextran increased in size 28-fold following autoclaving. The data show that following autoclaving, reduced dextran remains more tightly bound to the iron particle compared to native dextran.

A second type of increased stability achieved herein by use of reduced dextran to coat USPIO is the property of pH of the bulk solvent. The pH of USPIO coated with reduced dextran dropped 0.9 pH units following autoclaving, compared to a drop of 1.6 pH units for USPIO coated with native dextran.

Even greater stability to the autoclaving process was observed for particles coated with carboxymethyl reduced dextran compared to carboxymethyl native dextran. The data in Table 9 indicate that USPIO coated with carboxymethyl non-reduced native dextran showed a 10–50 fold increase in amount of particulate matter following autoclaving. In contrast, USPIO coated with carboxymethyl reduced dextran experienced no change in size or quantity of particulate matter upon autoclaving. Another indication of the stabilizing effect of the carboxymethyl reduced polysaccharides coating confer on the colloid suspension and bulk solvent was the stability of the solvent pH. The data in both Tables 8 and 9 show that the particles coated with reduced dextran had significantly improved pH stability upon autoclaving, compared to those coated with native dextran.

TABLE 8

Effect of autoclaving on pH, size, and bound polysaccharide of colloids coated with native and reduced dextran.

		pre autoclaved			post autoclaved[a]		
Example	dextran coating	pH	bound dextran g/g	MVD nm	pH	bound dextran g/g	MVD nm
29	native T10	7.0	0.79	21	5.5	0.56	587
23	reduced T10	7.4	1.26	18	6.7	0.96	23

[a]Samples were prepared at a concentration of 20 mg iron per ml and autoclaved for 30 minutes at 121° C.

TABLE 10

		Relaxivity				
Material	Coating	Susceptibility	MW (kDa)	R1	R2	R2/R1
Example 31	reduced carboxymethyl dextran	38,200	10	35.3	64.8	1.8
Combidex®	Dextran-	28,000	9.6	21.7	60.3	2.8
Gd-DTPA	T10	372		4.5	5.7	1.3

TABLE 9

Effect of autoclaving on pH, size, and particulates of colloids coated with carboxymethylated reduced and carboxymethylated native non-reduced dextran.

						particulates[a]			
Example	dextran	MVD		pH		>10 microns number/ml		>25 microns number/ml	
autoclaved	coating	pre	post	pre	post	pre	post	pre	post
30	CMD[b]	19	18	7.5	6.8	35	433	5	240
31	CMRD[c]	25	18	8.0	7.9	4	7	1	5

[a]Particulates were determined by USP analysis.
[b]CMD, carboxymethyl dextran (native)
[c]CMRD, carboxymethyl reduced dextran
[d]Samples were prepared at a concentration of 20 mg iron per ml and autoclaved for 30 minutes at 121° C.

Example 51

Procedures for Determining Relaxation Properties of Various Contrast Agents

Nuclear magnetic (NM) measurements (0.47T) were obtained in a Bruker Instruments pc120 table-top NM sample analyzer operating at 20 MHZ (Proton). Half a milliliter of each sample was placed in the 10 mm NM tubes for relativity measurements on the minispec. The placement of the sample in the sample chamber was optimized. The standards were run and their values recorded in the log.

Standard procedures were used for T1 and T2 determinations, and their values were recorded. T1 was measured using an inversion recovery technique. According to the IR technique, the sample is exposed to a 180° pulse and then a 90° pulse to put the magnetization in the plane of detection. After sampling; the time between the 180 and 90-degree pulses is changed, and sampled again. This is done for several durations. The resulting signals are governed by the equation $[M_\infty - M(t)]/M_\infty = (1-\cos\Theta)\exp(-t/T1)$. When a 3 parameter fit to data is performed, M_∞, Θ, and T1 are calculated.

T2 was measured using the CPMG technique, where a linear train of 180° pulses of variable length is provided to the sample. The amplitude of every second echo is measured. A fit is performed on the accumulated data using a two parameter (M_0 and T2) fit. Where $M(t)=M_0\exp(-t/T2)$, a plot of $\ln(M(t))$ versus t is linear with a slope of $-1/T2$. The inverse of the T1 and T2 was graphed with respect to the iron concentration of the sample. From the slope of best fit line the relaxivity was determined.

Example 52

Toxicity Studies in Rats. Toxicity of Reduced Dextran, Non-reduced Dextran, and CMRD Coated Colloids Administered in Vast Excess to Rats

An anaphylactic shock type of reaction to dextran can be exhibited by rats and by a small but significant fraction of the human population (Squire, J. R. et al., "Dextran, Its Properties and Use in Medicine," Charles C. Thomas, Springfield, Ill., 1955). The reaction resembles anaphylactic shock but does not require prior sensitization, and is characterized in rats by the rapid development of prostration, diffuse peripheral vasodilation, and edema of paws, snout and tongue (Voorhees, A. B. et al., *Proc. Soc. Exp. Biol. Med.* 1951, 76:254). When accompanied by barbiturate anesthesia, it produces marked hypotension and cyanosis (Hanna, C. H. et al., *Am. J. Physiol.* 1957, 191:615).

A procedure to measure the extent of rat paw edema response was employed to determine if the presence of reduced dextrans or their derivatives, rather than non-reduced native dextrans, in the coating of the iron oxide colloids could decrease or eliminate potential human adverse reactions upon intravenous injection. Rat paw edema was measured as the volume of the paw prior to and subsequent to injection of test material, using a plethysmometer, which is a differential volume measuring device. The dose of test material was injected, and a second reading was taken after a designated interval, and the per cent change in paw volume was calculated. The dose administered in these studies was 100 mg Fe/kg body weight, a dose much greater than that used as an imaging agent in rats, pigs, and humans (see Examples 53–56).

The results observed following administration of iron oxides coated with each of reduced and non-reduced T10

dextrans are shown in Table 11. A marked decrease in the edematous anaphylactic response was observed in those rats which were administered a USPIO preparation having the reduced dextran or reduced dextran derivatives as a coating, compared to those rats administered a USPIO preparation having a native non-reduced dextran coating.

TABLE 11

Effect of native and reduced polysaccharide coated particles on rat edema.

Example	coating and particle	% edema
29	native dextran coated USPIO	>50
23	reduced dextran coated USPIO	13
30	carboxymethyl native dextran coated USPIO	39
48	carboxymethyl reduced dextran non-magnetic colloid	12
31	carboxymethyl reduced dextran coated USPIO	0

The effect of the CMRD-USPIO preparations having increasing levels of carboxymethyl substitution on the extent of anaphylactic response, measured as percent edema, is shown in Table 12. The data show that a threshold level of substitution was necessary to reduce the edematous response, and that once this threshold of substitution was achieved, the decrease in response of the rats to dextran was a surprising elimination of the edematous response. That is, no edema was observed at 1,265 micromoles of carboxyl per gram.

TABLE 12

Extent of rat paw edema as a function of amount of carboxymethylation of dextran coating of USPIOs.

Example	micromol COOH per g dextran	% edema
32	110	24
33	130	54
34	280	81
35	450	37
36	580	105
31	1,265	0

Example 53

Toxicity Studies in Rats of Reduced and Non-reduced Dextrans

The procedure used in Example 52 was used to determine if the coating alone, that is, reduced dextrans or their derivatives rather than non-reduced native dextrans, could eliminate potential human adverse reactions upon intravenous injection. Rat paw edema was measured as the volume of the paw prior and subsequent to injection, as in Example 52. The dose administered in these studies was, as above, 100 mg test substance/kg body weight.

The results observed following administration of reduced and non-reduced T10 dextrans were similar for each material (Table 13). Reduced dextran T10 elicited the same extent of edema as native dextran T10. Elimination or decrease in edema could not be attributed merely to reduction of the dextran.

TABLE 13

Effect of native and reduced 10 kDa polysaccharides on rat edema showing more reduction has no significant effect

Example	test dextran	% edema
Dextran T-10 (commercial*)	native T10	61
3	reduced T10	67

*Obtained from Pharmacia-Upjohn (Piscataway, NJ)

Table 14 shows the effect of increased levels of carboxymethyl substitution of reduced dextran on the extent of anaphylactoid response, measured as percent edema. The data show that above a threshold level of carboxymethyl substitution, edema was decreased or eliminated. For dextrans above this threshold level of substitution, the decrease in the toxic response of the rats to dextran was a surprising elimination of response, that is, no edema was observed.

TABLE 14

Relationship between rat paw edema and degree of carboxymethylation of dextran T10 preparations.

Example	test substance	micromol COOH/ g per dextran	% edema
10	carboxymethyl reduced	110	65
12	carboxymethyl reduced	280	60
13	carboxymethyl reduced	450	56
5	carboxymethyl reduced	1,265	6
15	carboxymethyl reduced	1,887	1
16	carboxymethyl native	1,220	0

Example 54

Pharmacokinetics of CMRD Coated USPIO in the Rat: Blood Clearance

Three male CD® rats (Charles River Laboratories, Wilmington, Mass.; weight range 272 to 290 g) were anaesthetized intraperitoneally with a long lasting anesthetic, Inactin (100 mg per kg body weight). The femoral artery and vein were exposed by a small incision at the hip-femur joint, and the artery was cannulated with PE50 tubing connected to a 1 ml syringe filled with heparinized saline (10 units per ml). To serve as a baseline, 0.25 ml of arterial blood was collected at time zero, and CMRD coated USPIO (Example 31) was injected into the femoral artery. Blood samples of 0.25 ml were collected at the times indicated in FIGS. 4 and 5.

T2 magnetic relaxation times were measured in each sample, and the relaxivity (1/T2) was calculated. First-order reaction kinetics were used to determine the half-life of the sample in the blood ($t_{1/2}$). The equation used to fit the data was:

$$1/T2-1/T_{baseline}=Ae^{-kt}$$

where $1/T_2$ is the relaxivity of the blood at time t post-injection; $1/T_{baseline}$ is the baseline relaxivity, and Ae^{-kt} represents the first-order decay of the test material from the

blood. Taking the natural log of each side of this equation yields:

$$\ln (1/T2-1/T_{baseline}) = -kt + \ln A_0$$

According to this second equation, a graph of ln (1/T2−1/$T_{baseline}$) versus time, t, should give a straight line with slope −k (the first order rate constant) and intercept lnA_0 (which equals ln (1/T2−1/$T_{baseline}$ at time zero) if the rate of removal of the USPIO from blood follows first order kinetics. FIG. 5 shows that a straight line was obtained. The half-life ($t_{1/2}$), which is the time that the amount of CMRD coated USPIO decreased to one half its amount of concentration in the blood, was determined to be 67 min, with a range of 61 to 75 min at a confidence level of 95%.

Example 55

Magnetic Resonance Imaging Using CMRD Coated USPIO in the Rat

An MRI scan of a rat taken shortly after administration of 5 mg of CMRD coated USPIO (Example 31) per kg body weight is shown in FIG. 6B. The heart, aorta, and coronary artery were found to be readily imaged using this agent. An image of the rat taken pre-administration of the agent (FIG. 6A) is included to illustrate the substantial increase in contrast effected by administration of the test substance.

Example 56

MRI of CMRD Coated USPIO in the Pig

FIG. 7 illustrates enhanced MRI visualization of the heart and surrounding arteries, as well as the lungs and kidneys of the pig. Four doses of 0.4, 0.8, 1.6, and 2.2 mg of iron/kg body weight of sample (Example 31) were each administered to the pig in sequential order. Each dose was followed by administration of 20 ml of physiological saline, and an MRI image was obtained after each dose. The image shown in FIG. 7B is representative of images obtained after each administration. A preimage of the pig (FIG. 7A) is included to illustrate the substantial increase in contrast effected by the agent.

A problem associated with low molecular weight gadolinium based contrast agents is that they leak from the vascular space into the interstitial space and create a hazy background. This hazy background interferes with effective use of second or third injections of a contrast agent administered during a single examination. Such extravascular leakage might not be expected with carboxymethyl reduced dextran-coated USPIOs due to the relatively large size of the particle, compared to the size of the particles of a gadolinium contrast agent.

This expectation was confirmed by imaging of rats (Example 55) and in the data obtained by imaging of the pig (FIG. 7B). No background haze was observed following use of the CMRD USPIO compositions of the present invention. This observation enabled performance of additional vascular imaging tests, after sequential administration of additional doses. Upon intravenous administration, the CMRD coated USPIO, which is an embodiment of the invention, moved as a bolus rapidly into the arteries, organs, and veins, and achieved a uniform distribution in the blood after 20 minutes. Upon administration of a second bolus of the agent, additional good images were obtained. A third injection and a fourth injection were administered with similar results i.e., good images were obtained. Thus, the process of bolus injection and first pass application of the CMRD coated USPIO was demonstrated. Further, application of a multiple injection protocol within a reasonably short period of time after the first administration, the entire protocol being accomplished in a time period equivalent to a visit by a human subject to an imaging facility, was also demonstrated.

The principal advantages of capability of multiple bolus injections within a single examination are the opportunities to correct a deficiency in imaging that might arise after an injection, and to image multiple parts of the body during a single examination. In this manner, additional sites within the body of a subject can be imaged within a short period of time after scanning and analysis of earlier images from an earlier pass, and subsequent injections of contrast agent can be used to obtain different views, or to extend the view in one or more physical dimensions. For example, detailed analysis of the location and size of a blood clot in a limb such as a leg, can be performed using a series of views taken in the each of a first, second, and subsequent passes.

The capability for achieving additional multiple passes of administration of a composition of the invention and obtaining additional rounds of MRI data, beyond a first dose, present strong advantages of the compositions that are embodiments of the present invention. MRI analyses have in the past been limited by the physical length of the anatomical feature in need of imaging, and by the numbers of structures that can be imaged using a single detection instrument unit in a given time period.

The results obtained in pigs were observed also in human subjects (Examples 57 and 58).

Example 57

Intravenous Injection of CMRD Coated USPIO into Normal Human Subjects

The trial design employed thirty-five human subjects each administered one dose of CMRD T10 coated USPIO prepared according to Example 31(i.v.; 1–4 mg of iron/kg body weight). The objectives of Examples 57 and 58 were to examine subjects for any potential side effect of the treatment, to obtain data on the composition as an MRI contrast agent, and to determine the half-life of the composition in blood.

No adverse reactions attributable to administration of the composition were observed among the treated subjects at any dose, including the highest doses (4 mg/kg). For comparison, in clinical trials of Feridex I.V.®, approximately 2–3% of treated patients reported back pain, even though Feridex I.V.® and other comparable imaging products are administered in much smaller doses (e.g., 0.56 mg of iron/kg body weight) in order to minimize adverse events and obtain useful contrast. These data indicate that an effective dose of the CMRD coated USPIO particles of the invention is safer than an effective dose of a previously approved imaging agent, Feridex I.V.®

Example 58

Rapid Imaging Kinetics and Bio-distribution in Human Subjects

An initial intravenous bolus injection into human subjects of CMRD coated USPIO, prepared as in Example 31 yielded a bright MRI of the arterial portion of the circulatory system within 12 seconds post-administration (FIG. 8B). Following a further 15 seconds, MRI exposures yielded bright images of organs and veins. Equilibration of the agent throughout the vascular system was achieved within 20 minutes.

The organs capable of being imaged in the early phase following administration of the CMRD coated USPIO of the present invention included the heart, arteries and veins. Further, in addition to the larger elements of the circulatory system, the arterioles and venules of the extremities (fingers, toes) could be observed. This level of resolution allows applications to diagnosis of problems in circulation within the extremities, including the detection and localization of an area of phlebitis. Other organs that were readily imaged include the brain, kidneys, liver, spleen, and bone marrow. Lymph nodes could be imaged up to several hours after administration of an effective dose. The half-life of the agent in the blood was approximately observed to be 10–14 hours (see Table 15 and FIG. 9).

The particles ultimately were removed from circulation by being taken up by the reticuloendothelial system. During the presence of the composition at the early phase in the vascular system, and also in the late or post vascular phase in the reticuloendothelial system (RES), this composition was not observed to enter into interstitial spaces between cells. Thus, a hazy background, found to appear with usage of other compositions, for example, gadolinium based MR contract agents such as Magnevist® and DOTOREM®, is avoided during use of the CMRD-USPIO compositions, as synthesized by the methods of the Examples herein.

TABLE 15

Mean half-life of CMRD-USPIO T10
in human subjects as a function of dose.

Dose mg iron/kg	half-life, hours	standard deviation	# subjects
1	9.7	1.1	8
2	10.3	1.4	8
4	14.4	2.2	17

What is claimed is:

1. A method of providing an iron oxide complex for administration to a mammalian subject, the method consisting of:

producing a carboxyalkylated reduced polysaccharide iron oxide complex; and

sterilizing the complex by autoclaving.

2. A method according to claim 1, wherein the reduced polysaccharide is a reduced polymer of glucose.

3. A method according to claim 2, wherein the reduced polymer of glucose is a reduced dextran.

4. A method according to claim 1, wherein the reduced polysaccharide is produced by reacting a polysaccharide with a reagent selected from the group consisting of: a borohydride salt, and hydrogen in the presence of an hydrogenation catalyst.

5. A method according to claim 1, wherein producing the complex includes carboxyalkylating a reduced polysaccharide by carboxymethylation.

6. A method according to claim 5, wherein the reduced polysaccharide is a reduced dextran.

7. A method according to claim 6, wherein the administration to a mammalian subject is administration to a human.

8. A method according to claim 1, wherein the carboxyalkylated, reduced polysaccharide isolated as a sodium salt does not contain an infrared absorption peak in the region of about 1650 cm^{-1} to about 1800 cm^{-1}.

9. A method according to claim 1, wherein producing the carboxyalkylated reduced polysaccharide is achieved at a temperature of less than about 50° C.

10. A method according to claim 11, wherein producing the carboxyalkylated reduced polysaccharide is achieved at a temperature of less than about 40° C.

11. A method according to claim 1, wherein the iron oxide is superparamagnetic.

12. A reduced polysaccharide iron oxide complex produced according to the method of claim 1, wherein the produced complex is stable at a temperature of at least 100° C.

13. A reduced carboxyalkylated polysaccharide iron oxide complex wherein the produced complex is stable at a temperature of about 121° C.

14. A reduced polysaccharide iron oxide complex according to claim 13, wherein the produced complex is stable at a temperature of at least about 121° C. for a period of time effective to sterilize the complex.

15. A reduced polysaccharide iron oxide complex according to claim 14, wherein the carboxyalkylated reduced polysaccharide is selected from the group consisting of a carboxymethyl, carboxyethyl and carboxypropyl reduced polysaccharide.

16. A reduced polysaccharide iron oxide complex according to claim 15, wherein the reduced polysaccharide is a reduced dextran.

17. A reduced polysaccharide iron complex according to claim 15, wherein the carboxyalkylated reduced dextran is a carboxymethyl reduced dextran.

18. A reduced polysaccharide iron oxide complex according to claim 16, wherein the carboxyalkylated reduced dextran comprises at least about 750 micromole of carboxyl groups per gram of polysaccharide.

19. A reduced polysaccharide iron oxide complex according to claim 18, wherein the carboxyalkylated reduced dextran comprises at least about 900 micromole of carboxyl groups per gram of polysaccharide.

20. A reduced polysaccharide iron oxide complex according to claim 19, wherein the carboxyalkylated reduced dextran comprises at least about 1100 micromole of carboxyl groups per gram of polysaccharide.

21. A reduced polysaccharide iron oxide complex according to claim 20, wherein the carboxyalkylated reduced dextran comprises less than about 1500 micromole of carboxyl groups per gram of polysaccharide wherein said complex does not form substantial particulates.

22. A method of providing a contrast agent for in vivo MRI of a subject according to claim 1, consisting of the steps of:

formulating a composition which is a carboxymethylated reduced ultrasmall superparamagnetic iron oxide complex; and

terminally sterilizing the composition by autoclaving.

23. A method of providing a hematinic agent for treating a subject deficient in iron, consisting of the steps of:

formulating a composition which is a carboxymethylated reduced ultrasmall iron oxide complex; and

terminally sterilizing the composition by autoclaving.

24. A method according to claim 22 or 23, having the further step of providing the autoclaved composition in a unit dosage.

25. A reduced carboxyalkylated polysaccharide iron oxide complex which is stable at a temperature of about 121° C., wherein a sodium salt of the complex does not contain an infrared absorption peak in the region of about 1650 cm^{-1} to about 1800 cm^{-1}.

26. A reduced carboxyalkylated polysaccharide iron oxide complex according to claim 25, wherein the polysaccharide is carboxymethylated.

* * * * *

Attachment C

UNITED STATES PATENT AND TRADEMARK OFFICE

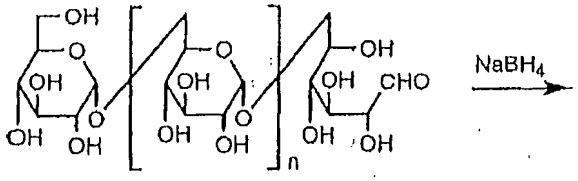
CERTIFICATE OF CORRECTION

PATENT NO. : 6,599,498 B1
DATED : July 29, 2003
INVENTOR(S) : Ernest V. Groman et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, Item [54] and Column 1, line 3,
Replace "CARBOHDRATE" with -- CARBOHYDRATE --

Column 7,
Scheme 1, lines 14-20, replace compound 2 with the following:



2

Column 13,
Line 21, replace "edemia" with -- edema --.

Signed and Sealed this

Second Day of March, 2004

JON W. DUDAS
Acting Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,599,498 B1
APPLICATION NO. : 09/521264
DATED : July 29, 2003
INVENTOR(S) : Ernest V. Groman et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Col. 13, line 17
replace "symtoms"
with --symptoms--.

In Col. 13, line 22
replace "edemia"
with --edema--.

In Col. 37, claim 10, line 65
replace "claim 11"
with --claim 9--.

In Col. 38, claim 13, line 8
replace "produced"
with --reduced--.

In Col. 38, claim 14, line 11
replace "produced"
with --reduced--.

In Col. 38, claim 22, line 46
insert --polysaccharide--
immediately after "reduced".

In Col. 38, claim 23, line 50
insert --according to claim 1,--
immediately after "iron,".

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,599,498 B1
APPLICATION NO. : 09/521264
DATED : July 29, 2003
INVENTOR(S) : Ernest V. Groman et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Col. 38, claim 23, line 52
insert --polysaccharide--
immediately after "reduced".

Signed and Sealed this

Twenty-seventh Day of January, 2009

John Doll

JOHN DOLL
Acting Director of the United States Patent and Trademark Office

Attachment D

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MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,599,498	$900.00	$0.00	12/01/06	09/521,264	07/29/03	03/08/00	04	NO	1275/190

Attachment E

NDA 22-180 - Submissions Log

(Feraheme™ for the treatment of iron deficiency anemia in adult patients with chronic kidney disease)

Date	Submission	Type	Description
6/30/2009			**NDA Approval Letter received**
6/18/2009	0030	Edits to the PI	Revision to draft Prescribing Information
6/16/2009	0029	Edits to the Labels	Revision to draft Carton and Container Labels
6/10/2009	0028	Edits to the PI	Revision to draft Prescribing Information
6/9/2009	0027	Response to IR	Post-marketing Commitment Study synopsis and timelines
6/4/2009	0026	Response to IR and Edits to the PI	Revision to draft Prescribing Information and Pediatric Post-marketing Requirement timelines
5/26/2009	0025	Response to IR and Edits to the PI	Revision to draft Prescribing Information
5/5/2009	Copy to NDA Review Division	Submission of Tradename to DMEPA	Tradename was submitted to DMEPA for final review
4/29/2009	Resubmission	Resubmission	Complete Response to PDUFA Action letter dated Dec. 22, 2008
			Informed AMAG plans for Resubmission to PDUFA Action letter dated Dec. 22, 2008
4/14/2009	Copy to NDA Review Division	Submission to FDA-NE District Office	Follow-up Response to 483 Observations
4/8/2009	Copy to NDA Review Division	Submission to FDA-NE District Office	Follow-up Response to 483 Observations
3/30/2009	0024	Response to Information Request (IR)	Revision to draft Carton and Container Labels
3/20/2009	Copy to NDA Review Division	Submission to FDA-NE District Office	Follow-up Response to 483 Observations and Regulatory Meeting of March 11, 2009
2/10/2009	0023	Response to	Revision to draft Carton and Container Labels, Addition of

		IR	new Labeling vendor
1/7/2009	0022	Response to IR	Post-marketing studies timelines, Edits to Package Insert, CMC information
12/17/2008	General	Email	Confirmation of Teleconference to discuss the pending action of the NDA resubmission
11/10/2008	Copy to NDA Review Division	Submission to FDA-NE District Office	Follow-up Response to 483 Observations
11/3/2008	Copy to NDA Review Division	Submission to FDA-NE District Office	Response to 483 Observations
10/30/2008	Resubmission	Resubmission	Complete Response to PDUFA Action letter dated Oct. 17, 2008
10/3/2008	0021	Response to IR	Clinical information on injectable iron naïve patients
10/1/2008	0020	Response to IR	Revision to draft Carton and Container Labels
9/25/2008	0019	Response to IR	Revision to draft Prescribing Information
9/23/2008	0018	Response to IR	Clinical, CMC and Labeling questions
9/22/2008	0017	Response to IR	Revision to draft Carton and Container Labels; additional Proposed Proprietary Names
9/5/2008	0016	Response to IR	CMC information and revisions to Carton and Container Labels
9/3/2008	0015	General	Notification of AMAG address change to 100 Hayden Avenue, Lexington, MA
	0014	Response to IR	Additional information for the proposed Pediatric studies
8/7/2008	0013	Response to IR	CMC information
8/5/2008	0012	Response to IR	Draft Prescribing Information in MS Word format
8/4/2008	0011	Response to IR	Information on proposed Pediatric studies
7/24/2008	0010	Response to IR	Clinical Study Sites information for GCP inspection
7/16/2008	0009	Labeling	Submission of draft Physician Sample Labels and Physician Sample Kit

7/14/2008	0008	Response to IR	Details on Educational Programs and Risk Minimization Tools proposed in the NDA
6/23/2008	0007	Response to IR	Information on Ferritin, TSAT and Phosphate levels
6/5/2008	0006	Response to IR	Information on Treatment Discontinuations in the clinical studies
5/20/2008	0005	Response to IR	Information on Hypersensitivity/Allergic Reactions related to adverse events in the clinical studies
4/28/2008	0004	Clinical	120 Day Safety Update Report
4/14/2008	0003	Response to IR	Information on Clinical Pharmacology
4/10/2008	0002	Proprietary Names	Request for Review of Proposed Proprietary Names
4/3/2008	0001	General	Responses to 74 Day Letter and comments from NDA Orientation Presentation
2/27/2008	Email	Slide presentation	Copy of the presentation for NDA 22-180 Application Orientation Meeting
12/18/2007	**0000**	**General**	**NDA 22-180 Original Application**

NDA 22-180 - DDMAC Submissions Log

Feraheme™

Date	Type of submission	Description
6/30/2009	DDMAC - final	Press Release & Now Approved Website
6/18/2009	DDMAC - Advisory Comment	Supplement to DRAFT Press Release
6/10/2009	DDMAC - Advisory Comment	DRAFT Press Release

IND 62,745 - Submissions Log

(Feraheme™ Iron therapy)

Date	Submission or Correspondence (Type)	Serial #	Description
5/4/2009	Submission (follow-up safety report)	0120	Follow Up #1 to Report Mfr. # 2009PAD001000002
4/16/2009	Submission (other)	0119	Updated Investigator's Brochure dated Jan. 12, 2009
4/1/2009	Submission (initial safety report)	0118	15 Day Safety Report for Mfr. # 2009PAD001000002
2/17/2009	Correspondence (meeting minutes from FDA)		Minutes from FDA from Type C Meeting held Feb. 5, 2009 to discuss development program in cancer patients
1/5/2009	Submission (other)	0117	Briefing Package for Type C Meeting scheduled on Feb. 5, 2009 to discuss development program in cancer patients
12/3/2008	Correspondence		Letter from FDA granting Type C Meeting for Feb. 5, 2009 to discuss development program in cancer patients
11/26/2008	Submission (other)	0116	Type C Meeting Request for discussion of development program in cancer patients
11/13/2008	Correspondence		FDA comments on protocols AUB-001 and AUB-002
10/16/2008	Submission (Annual report)	0115	2008 Annual Report
9/12/2008	Submission (follow-up safety report)	0114	15 Day IND Safety Report for Mfr Report # AMAG-2008-0002
9/4/2008	Correspondence (initial safety report)	N/A	7 Day IND Safety Report faxed for Mfr Report # AMAG-2008-0002
9/3/2008	Submission (other)	0113	Notification to FDA of AMAG move (Corporate Offices only) to 100 Hayden Avenue, Lexington, MA
7/2/2008	Submission (other)	0112	Updated Investigator's Brochure dated June 30, 2008
6/19/2008	Submission (New protocol)	0111	Submission of draft AUB protocols FER-AUB-001 and 002 for comments
3/27/2008	Correspondence		FDA comments on AUB protocols that were submitted on March 12, 2008

Date	Submission or Correspondence (Type)	Serial #	Description
3/12/2008	Submission (other)	0110	Request for Agency feedback on Clinical Development Plan in women with AUB
2/26/2008	Submission (other)	0109	AMAG version of minutes from Feb. 19, 2008 Type C meeting held to discuss the clinical development program for the treatment of IDA in women with AUB
2/26/2008	Correspondence (meeting minutes)		Minutes from FDA for Feb. 19, 2008 Type C meeting held to discuss the clinical development program for the treatment of IDA in women with AUB
2/13/2008	Correspondence (meeting minutes)		FDA pre-meeting comments for Feb. 19, 2008 Type C meeting scheduled to discuss the clinical development program for the treatment of IDA in women with AUB
1/21/2008	Submission (other)	0108	Briefing Package for Type C meeting scheduled to discuss the clinical development program for the treatment of IDA in women with AUB
12/13/2007	Correspondence		Notification that Type C meeting is granted to discuss AUB indication on Feb. 21, 2008
12/7/2007	Submission (meeting request)	0107	Request for Type C Meeting to discuss the clinical development plan in women with Abnormal Uterine Bleeding (AUB)
10/18/2007	Submission (annual report)	0106	2007 Annual Report
9/14/2007	Submission (follow-up safety report)	0105	Final MedWatch Form for Mfr. No. AMI000217
9/14/2007	Submission (follow-up safety report)	0104	Final MedWatch Form for Mfr. No. AMI000226
9/14/2007	Submission (follow-up safety report)	0103	Final MedWatch Form for Mfr. No. AMI000228
9/14/2007	Submission (follow-up safety report)	0102	Final MedWatch Form for Mfr. No. AMI000225
9/14/2007	Submission (follow-up safety report)	0101	Final MedWatch Form for Mfr. No. AMI000229
8/1/2007	Correspondence (meeting minutes)		Minutes from FDA from Pre-NDA meeting held on July 20, 2007
7/31/2007	Submission (Company name change notification)	0100	Letter informing FDA of company name change to AMAG Pharmaceuticals, Inc.

Date	Submission or Correspondence (Type)	Serial #	Description
7/18/2007	Correspondence		FDA pre-meeting response to questions submitted for the July 20, 2007 Pre-NDA meeting
6/21/2007	Submission (meeting information package)	0099	Pre-NDA Meeting Briefing Package
6/5/2007	Submission (follow-up safety report)	0098	Final MedWatch Form for Mfr. No. AMI000231.
5/25/2007	Submission (other)	0097	Updated Investigator's Brochure, dated May 22, 2007
5/17/2007	Correspondence (meeting notification)		Pre-NDA Meeting scheduled for July 20, 2007
5/15/2007	Submission (meeting request)	0096	Pre-NDA Meeting Request
5/15/2007	Submission (CMC amendment)	0095	Information on ferumoxytol commercial configuration packaging
5/11/2007	Submission (response to Information request)	0094	Additional details for a previously submitted safety report
5/3/2007	Submission (initial safety report)	0093	15-day Safety Report for Mfr# AMI000231
5/1/2007	Submission (response to Information request)	0092	Submission regarding Phase III study results per agency's request
4/20/2007	Submission (initial safety report)	0091	15-day Safety Report for Mfr# AMI000228
4/18/2007	Submission (initial safety report)	0090	15-day Safety Report for Mfr# AMI000229
3/26/2007	Submission (initial safety report)	0088	15-day Safety Report for Mfr# AMI000226
3/26/2007	Submission (initial safety report)	0087	15-day Safety Report for Mfr# AMI000225
3/21/2007	Correspondence (Information request from FDA)		FDA request for additional information for previously submitted safety report and on results of ferumoxytol clinical studies
3/8/2007	Submission (follow-up safety report)	0086	Follow up to Mfr# AMI000217.

Date	Submission or Correspondence (Type)	Serial #	Description
2/23/2007	Submission (initial safety report)	0085	15-day Safety Report for Mfr# AMI000221
1/29/2007	Submission (initial safety report)	0083	15-day Safety Report for Mfr# AMI000217
1/26/2007	Submission (response to information request)	0081	Additional information for a previously submitted safety report
1/19/2007	Correspondence (information request)		Additional information on previously submitted safety report requested
1/16/2007	Correspondence (information request)		FDA requested additional information regarding the safety data listed in the annual report
1/12/2007	Correspondence (information request)		FDA requested additional information for a previously submitted safety report
12/21/2006	Submission	0079	Annual Report Safety Data - update
12/6/2006	Submission (follow-up safety report)	0078	Re-classification of previously submitted SAE – Mfr# AMI000073 and AMI000072
11/28/2006	Submission (follow-up safety report)	0077	Re-classification of previously submitted SEA – Mfr# AMI000080
11/21/2006	Submission (pharm/tox amendment)	0076	Nonclinical study report
11/15/2006	Submission (CMC amendment)	0074	Stability Data for ferumoxytol from 1998 through June 2006
10/27/2006	Correspondence (tel)		FDA confirmed that the proposed Phase III clinical plan seems OK as long as there are no unexpected safety signals identified in clinical trials
10/13/2006	Submission (pharm/tox amendment)	0072	Nonclinical study reports
9/29/2006	Submission	0071	Final Clinical Study Reports for Study 62,745-3 and Study 62,745-4
9/20/2006	Submission (annual report)	0069	Annual Report - 2006
8/24/2006	Submission (follow-up safety report)	0068	7 Day IND Safety Report. Mfr. No. AMI000175
8/21/2006	Submission (initial safety report)		Fax to FDA; 7 Day IND Safety Report: Hypotension in Subject #533 in Study 62,745-5

Date	Submission or Correspondence (Type)	Serial #	Description
8/21/2006	Correspondence (safety event)		Notification of a Serious Adverse Event
7/31/2006	Submission (initial safety report)	0065	Safety Report
7/31/2006	Submission (protocol amendment)	0066	62,745-9 protocol amendment #2
6/28/2006	Submission (protocol amendment)	0064	Phase III amended protocols and statistical analysis plans
6/6/2006	Correspondence		FDA comments on protocol 62745-9
5/30/2006	Submission (protocol amendment)	0062	Amendment to Protocol 62,745-9
5/3/2006	Submission	0060	Information on non-expedited SAEs
5/3/2006	Correspondence		Information on non-expedited SAE
4/20/2006	Submission (pharm/tox amendment)	0058	Nonclinical study report
4/18/2006	Submission (new protocol)	0059	Submission of Protocol 62,745-9
3/2/2006	Correspondence		Minutes from FDA from Jan. 17, 2006 meeting
1/9/2006	Correspondence		Supplemental questions for feedback at the scheduled Jan. 17, 2006 meeting
12/29/2005	Submission	0055	Pre-meeting package to support Jan. 17, 2006 meeting
12/21/2005	Correspondence		FDA feedback on efficacy endpoints in Phase III studies and protocol amendments
11/21/2005	Correspondence		T-Con with FDA of Phase III CKD Studies
11/17/2005	Submission (protocol amendment)	0053	Phase III Protocols amendments
11/17/2005	Correspondence		Confirmation from FDA of Jan. 17, 2006 meeting
11/15/2005	Submission (protocol amendment)	0052	Phase III Protocols amendments
11/3/2005	Submission (meeting request)	0051	Type A Meeting Request

Date	Submission or Correspondence (Type)	Serial #	Description
10/17/2005	Correspondence		FDA declined the meeting request but agreed to provide written feedback to protocol amendments
10/12/2005	Submission (CMC amendment)	0050	Additional copies of briefing package for Type C meeting to be held on Nov. 4, 2005
9/30/2005	Submission (CMC amendment)	0049	Briefing package for Type C meeting to be held on 4 Nov 2005
9/29/2005	Submission (meeting request)	0048	Meeting request to discuss the Phase III protocol amendments
9/27/2005	Submission (follow-up safety report)	0047	Follow up to Mfr. No. AMI000080
9/13/2005	Submission (follow-up to safety report)	0046	Follow up to Mfr. No. AMI000072
9/9/2005	Submission (initial safety report)	0045	15-day Safety Report for Mfr# AMI000080
8/25/2005	Submission (annual report)	0044	Annual Report - 2005
8/19/2005	Submission	0043	Draft Clinical Study Reports for Studies 62745-3 and 62745-4
8/15/2005	Submission (initial safety report)	0042	15-day Safety Report for Mfr# AMI000073 and AMI000072
7/19/2005	Submission (meeting request)	0041	Request for Type C Meeting - CMC discussion
7/11/2005	Correspondence		FDA Minutes from June 21, 2005 meeting
6/24/2005	Submission	0040	Correspondence on FDA meeting held June 21, 2005.
6/21/2005			FDA Meeting on efficacy analysis & stat plan for Phase III pivotal studies
6/20/2005	Correspondence		FDA pre-meeting response to questions to be discussed at June 21, 2005 meeting
6/15/2005	Submission	0039	Submission of PK Study proposal
5/19/2005	Submission (meeting package)	0038	Briefing Package for June 21, 2005
3/16/2005	Submission (pharm/tox amendment)	0035	Nonclinical study reports
3/11/2005	Submission (meeting request)	0034	Request for a Meeting to review Phase III study endpoints and statistical analysis plans
12/10/2004	Submission (protocol amendment)	0033	Amendment to Protocol 62745-5

Date	Submission or Correspondence (Type)	Serial #	Description
9/8/2004	Submission (protocol amendment)	0029	Amendment to 62745 -8 Protocol
8/20/2004	Submission (pharm/tox amendment)	0028	Nonclinical study protocols
8/19/2004	Submission (annual report)	0027	Annual Report - 2004
8/5/2004	Submission (protocol amendments)	0022	Revisions to Protocols -5, -6 and -7
5/3/2004	Submission (pharm/tox amendment)	0020	Nonclinical study reports
4/2/2004	Submission (new protocols)	0017	FINAL protocols submitted for Phase 2 studies: 62,745 -5, -6, -7 and -8.
1/29/2004	Submission	0015	Draft clinical protocols and stat plan (Protocols 62,745-5, -6, -7, & -8)
11/7/2003	Correspondence		Minutes from FDA for Oct. 9, 2003 meeting
10/21/2003	Correspondence		Meeting responses/overheads from Oct. 9, 2003 meeting with FDA
10/2/2003	Correspondence		FDA pre-meeting response to questions submitted in Briefing Package for Oct. 9, 20 03 meeting
9/10/2003	Submission (meeting package)	0014	Briefing Package for FDA meeting scheduled on Oct. 9, 2003
8/26/2003	Correspondence		Meeting Confirmation for Oct. 9, 2003
8/5/2003	Submission (meeting request)	0013	Meeting Request - End of Phase II
7/24/2003	Submission	0012	Annual Report - 2003
4/21/2003	Submission (safety report)	0011	Report of subject death in study 62,745-3 (unrelated to study drug)
1/15/2003	Submission (initial safety report)	0009	SAE notification
9/13/2002	Submission (annual report)	0008	Annual Report 2002
7/18/2002	Submission (new protocols)	0007	Final Phase 2 study Protocols
4/30/2002	Submission (Pharm/tox and clinical amendment)	0006	Nonclinical study (interim) reports; Clinical study reports; additional details on ferumoxytol dosing

Date	Submission or Correspondence (Type)	Serial #	Description
3/14/2002	Submission (protocol amendment)	0005	Amendment to Study 62745-2 protocol
11/2/2001	Correspondence		Safety update on the first 5 patients dosed in the 62,745-2 study; no AEs reported
10/2/2001	Submission (new clinical protocol)	0004	Protocol for Phase I study 62,745-2
7/13/2001	Submission (protocol amendment)	0003	Amendment to Study 62,745-2 protocol
7/13/2001	Correspondence		FDA confirmation for initiation of Study 62,745-2
7/12/2001	Correspondence		Discussion with FDA on ferumoxytol dosing and administration in Study 62,745-2
7/12/2001	Correspondence		Submission of study 62,745-2 details
7/11/2001	Submission	0002	Dosing details for single dose study
7/11/2001	Correspondence (response to information request)		Details on planned nonclinical studies in support of ferumoxytol dosing in clinical studies provided
7/11/2001	Correspondence		Teleconference with FDA regarding nonclinical studies in support of ferumoxytol dosing in clinical studies
6/29/2001	Correspondence		Letter from FDA acknowledging receipt of IND 62,745
6/26/2001	Submission	0001	Final reports and new protocols for nonclinical studies
6/14/2001	Submission	0000	Initial IND Submission
Note: The following serial numbers contain the submission of FDA Forms 1572 & CVs for New Investigators: 0089, 0084, 0082, 0080, 0075, 0073, 0070, 0067, 0063, 0061, 0057, 0056, 0054, 0037, 0036, 0032, 0031, 0030, 0026, 0025, 0024, 0023, 0021, 0019, 0018, 0016, 0010.			

IND 58,254 – Submissions Log

(Feraheme™ Imaging)

Date	Submission or Correspondence (Type)	Serial #	Description
5/4/2009	Submission (follow-up safety report)	0053	Follow Up Safety Report for Mfr. # 2009PAD001000002
4/17/2009	Submission (protocol amendment)	0051	Protocol Amendment for FER-PAD-001
4/1/2009	Submission (initial safety report)	0050	15 Day Safety Report for Mfr. # 2009PAD001000002
3/10/2009	Submission (other)	0048	Submission of DSMB Charter for Study FER-PAD-001
10/16/2008	Submission (annual report)	0043	2008 Annual Report
9/12/2008	Submission (follow-up safety report)	0041	15 Day IND Safety Report from Investigator Sponsored Study
9/4/2008	Correspondence (initial safety report)		7 Day Safety Report Mfr Report # AMAG-2008-0002
9/3/2008	Submission	0040	Notification on Change of Company Corporate Address to Lexington, MA
8/19/2008	Correspondence		FDA Letter granting Fast Track Approval for PAD program
7/3/2008	Submission (new protocol)	0039	New clinical Protocol FER-PAD-001 and an updated Investigator Brochure
6/19/2008	Submission	0038	Request for Fast Track Designation for ferumoxytol VE-MRI program
5/30/2008	Submission	0037	Resubmission of request for Fast Track designation
5/16/2008	Correspondence		Letter from FDA regarding request for Fast Track designation for ferumoxytol PAD program
3/24/2008	Submission	0036	Request for Fast Track Designation for ferumoxytol Vascular Enhanced Magnetic Resonance Imaging (VE-MRI) program

Date	Submission or Correspondence (Type)	Serial #	Description
3/13/2008	Submission (safety reports)	0035	Mfg reports: AMI000175 ; AMI000080; AMI000072 ; and AMI000073
8/13/2007	Submission (annual report)	0034	2007 Annual Report
7/31/2007	Submission	0033	Change in company name to AMAG Pharmaceuticals
5/29/2007	Submission	0032	Updated Investigator Brochure (May 22, 2007)
3/30/2007	Submission (pharm/tox amendment)	0031	Nonclinical study report
3/26/2007	Submission (pharm/tox amendment)	0030	Nonclinical study reports
3/23/2007	Submission (CMC amendment)	0029	Stability data update
2/9/2007	Submission (CMC amendment)	0027	Stability data update
2/9/2007	Submission (pharm/tox amendment)	0028	Nonclinical study reports
8/2/2006	Submission (initial safety report)	0026	Safety Reports from 62,745 -9 study
7/24/2006	Submission (annual report)	0025	2006 Annual Report
4/20/2006	Submission (pharm/tox amendment)	0024	Nonclinical study reports
2/22/2006	Submission	0022	Copy to FDA on safety information notification to clinical sites
8/25/2005	Submission (annual report)	0020	2005 Annual Report
8/16/2005	Submission (initial safety report)	0019	Safety reports for Mfr# AMI000073 and AMI000072
3/14/2005	Submission (pharm/tox amendment)	0018	Nonclinical study reports
9/20/2004	Submission (annual report)	0016	2004 Annual Report

Date	Submission or Correspondence (Type)	Serial #	Description
5/3/2004	Submission (pharm/tox amendment)	0015	Nonclinical Study reports
7/3/2003	Submission (annual report)	0014	2003 Annual Report
9/12/2002	Submission (annual report)	0012	2002 Annual Report
8/16/2002	Submission (protocol amendment)	0011	Amendment for Study 58,254-5 protocol
3/8/2002	Submission (new protocol)	0009	Study 58254-5 protocol
12/11/2001	Submission (new protocol)	0008	Study 58,254-4 protocol
7/19/2001	Submission (annual report)	0007	2001 Annual Report
9/23/2000	Correspondence		FDA comments on nonclinical studies
8/22/2000	Submission (new protocol)	0006	Study 58,254-2 protocol
7/21/2000	Submission (Pharm/tox amendment)	0005	Nonclinical study protocols
7/5/2000	Submission (annual report)	0004	2000 Annual Report
10/14/1999	Submission (response to information request)	0003	Response to Pharm/Tox questions from FDA
7/21/1999	Submission (protocol amendment)	0001	Amendment to Study 7228-01 protocol
7/1/1999	Correspondence		FDA comments on nonclinical program, CMC and clinical protocol
5/20/1999	Correspondence		Additional CMC information
5/11/1999	Correspondence		Acknowledgement for IND submission
5/5/1999	Submission (initial IND)	0000	Original IND submitted

Note: The following serial numbers contain the submission of FDA Forms 1572 & CVs for New Investigators: 0054, 0052, 0049, 0047, 0046, 0045, 0044, 0042, 0023, 0021, 0017, 0013, 0010, 0002.

Attachment F

ASSIGNMENT

ASSIGNOR: Ernest V. Groman
Kenneth G. Paul
Timothy B. Frigo
Howard Bengele
Jerome M. Lewis

ASSIGNEE: Advanced Magnetics, Inc.
61 Mooney Street
Cambridge, MA 02138

STATE OF INCORPORATION OF ASSIGNEE: Delaware

INVENTION: HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES

ATTORNEY DOCKET: 1275/190

SERIAL NO: Not Yet Assigned

FILED: Herewith

Assignor is the sole inventor (if only one inventor is listed above) or a joint inventor (if more than one inventor is listed above) of the above invention (the "Invention") described in a United States patent application (the "Application") bearing the above attorney docket number and having as a title the above name for the Invention. The Application has a Patent and Trademark Office filing date and serial number as indicated above, or if no filing date and serial number are shown, has a Declaration executed by Assignor contemporaneously with this Assignment.

For valuable consideration, receipt of which is acknowledged, each Assignor hereby assigns to Assignee (which term shall include Assignee's successors and assigns), all of Assignor's right, title and interest in the Invention, all improvements therein, the Application and

all priority rights arising therefrom, and any patents, and any reissues and extensions thereof, which issue in any country upon any patent applications which correspond with any of the following: the Application, any divisional, continuation-in-whole, or substitute United States application which is based on the Application; or any continuation-in-part United States application (including divisions, continuations-in-whole or -in-part, and substitutions thereof or therefor) based in-part on any of the above described applications.

Each Assignor further agrees that such Assignor and Assignor's heirs and legal representatives will, without further consideration, cooperate with Assignee in the prosecution of all of the above applications, execute, verify, acknowledge and deliver all such further papers, including applications for patents and for reissues and extensions therefor, and instruments of assignment and transfer thereof, and will communicate any facts known to Assignor relating to the Invention, to obtain or maintain or enforce patents for the Invention and improvements therein in any and all countries and to vest title thereto in Assignee. Each Assignor further agrees that such Assignor will, without further compensation to Assignor during the term of such Assignor's employment by Assignee and thereafter for reasonable compensation as determined by Assignee, perform such other acts as may be reasonably required when requested by Assignee, including attending depositions, preparing and executing declarations and affidavits and testifying as a witness, to obtain or maintain or enforce patents for the Invention and improvements therein in any and all countries and to vest title thereto in Assignee.

IN WITNESS WHEREOF, each Assignor hereby executes this instrument on the date set forth below.

Date: 6 MARCH 2000

Ernest V. Groman, Assignor

STATE OF Mass)
) ss.
COUNTY OF Middlesex)

Before me, a notary public in and for said county and state, personally appeared Ernest V. Groman, to me known to be the person described in the foregoing instrument, who, being first duly sworn, acknowledged his signature on the foregoing instrument in my presence and declared the same to be his free act and deed on the date written above opposite his signature.

Notary Public
My Commission Expires: May 18, 2001

(seal)

Date: 3/3/00

Kenneth G. Paul, Assignor

STATE OF MASSACHUSETTS)
) ss.
COUNTY OF MIDDLESEX)

Before me, a notary public in and for said county and state, personally appeared Kenneth G. Paul, to me known to be the person described in the foregoing instrument, who, being first duly sworn, acknowledged his signature on the foregoing instrument in my presence and declared the same to be his free act and deed on the date written above opposite his signature.

Notary Public
My Commission Expires: MY COMMISSION EXPIRES 2-5-2004

(seal)

Date: 3-3-00

Timothy B. Frigo

Timothy B. Frigo, Assignor

STATE OF MASSACHUSETTS)
) ss.
COUNTY OF MIDDLESEX)

Before me, a notary public in and for said county and state, personally appeared Timothy B. Frigo, to me known to be the person described in the foregoing instrument, who, being first duly sworn, acknowledged his signature on the foregoing instrument in my presence and declared the same to be his free act and deed on the date written above opposite his signature.

Marlene Kaplan Goldstein

Notary Public
My Commission Expires: 4/7/00

(seal)

Date: 3-3-00

Jerome M Lewis

Jerome M. Lewis, Assignor

STATE OF MASSACHUSETTS)
) ss.
COUNTY OF MIDDLESEX)

Before me, a notary public in and for said county and state, personally appeared Jerome M. Lewis, to me known to be the person described in the foregoing instrument, who, being first duly sworn, acknowledged his signature on the foregoing instrument in my presence and declared the same to be his free act and deed on the date written above opposite his signature.

Marlene Kaplan Goldstein

Notary Public
My Commission Expires: 4/7/00

(seal)

110746

Date: 3-3-00

Howard H. Bengele

Howard H. Bengele, Assignor

STATE OF MASSACHUSETTS)
) ss.
COUNTY OF MIDDLESEX)

Before me, a notary public in and for said county and state, personally appeared Howard H. Bengele, to be know to be the person described in the foregoing instrument, who, being first duly sworn, acknowledged his signature on the foregoing instrument in my presence and declared the same to be his free act and deed on the date written above opposite his signature.

Notary Public
My Commission Expires: 4/7/00

(seal)

Attachment G

1275/190

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

JUNE 08, 2000

DOCKETED
RECEIVED
JUN 19 2000
BROMBERG & SUNSTEIN

101311670A

BROMBERG & SUNSTEIN LLP
SONIA K. GUTERMAN
125 SUMMER STREET
BOSTON, MA 02110

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 03/08/2000

REEL/FRAME: 010682/0276
NUMBER OF PAGES: 6

BRIEF: ASSIGNMENT OF ASSIGNOR''S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
GROMAN, ERNEST V. DOC DATE: 03/06/2000

ASSIGNOR:
PAUL, KENNETH G. DOC DATE: 03/03/2000

ASSIGNOR:
FRIGO, TIMOTHY B. DOC DATE: 03/03/2000

ASSIGNOR:
BENGELE, HOWARD DOC DATE: 03/03/2000

ASSIGNOR:
LEWIS, JEROME M. DOC DATE: 03/03/2000

ASSIGNEE:
ADVANCED MAGNETICS, INC.
61 MOONEY STREET
CAMBRIDGE, MASSACHUSETTS 02138

04-10-2000

Docket No.: 1275/190

FORM PTO-1595 (Modified)
(Rev. 6-93)
OMB No. 0651-0011 (exp.4/94)
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P08/REV02

EET

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

101311670

Tab settings → → → ▼ ▼ ▼ ▼ ▼

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):
Ernest V. Groman
Kenneth G. Paul
Timothy B. Frigo
Howard Bengele
Jerome M. Lewis

Additional names(s) of conveying party(ies) ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: Advanced Magnetics, Inc.

Internal Address:

Street Address: 61 Mooney Street

City: Cambridge State: MA ZIP: 02138

Additional name(s) & address(es) attached? ☐ Yes ☒ No

JC586 U.S. PTO
09/521264
03/08/00

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other

Execution Date: March 3, 2000 and March 6, 2000

4. Application number(s) or registration numbers(s): 09521264 3.8.00

If this document is being filed together with a new application, the execution date of the application is: March 8, 2000

A. Patent Application No.(s)

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Sonia K. Guterman

Internal Address: BROMBERG & SUNSTEIN LLP

Street Address: 125 Summer Street

City: Boston State: MA ZIP: 02110

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):.....$ 40.00

☒ Enclosed - Any excess or insufficiency should be credited or debited to deposit account

☐ Authorized to be charged to deposit account

8. Deposit account number:

19-4972

04/10/2000 DNGUYEN 00000284 09521264

DO NOT USE THIS SPACE

01 FC:581 40.00 OP

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Sonia K. Guterman — Sonia K. Guterman — March 8, 2000

Name of Person Signing — Signature — Date

Total number of pages including cover sheet, attachments, and document: 6

Attachment H

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF OWNERSHIP, WHICH MERGES:

"AMAG NAME CHANGE SUB, INC.", A DELAWARE CORPORATION,

WITH AND INTO "ADVANCED MAGNETICS, INC." UNDER THE NAME OF "AMAG PHARMACEUTICALS, INC.", A CORPORATION ORGANIZED AND EXISTING UNDER THE LAWS OF THE STATE OF DELAWARE, AS RECEIVED AND FILED IN THIS OFFICE THE TWENTY-FOURTH DAY OF JULY, A.D. 2007, AT 8:44 O'CLOCK A.M.

0926007 8100M

070848306

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 5868772

DATE: 07-24-07

State of Delaware
Secretary of State
Division of Corporations
Delivered 08:51 AM 07/24/2007
FILED 08:44 AM 07/24/2007
SRV 070843848 - 0926007 FILE

CERTIFICATE OF OWNERSHIP AND MERGER OF

AMAG Name Change Sub, Inc.,
a Delaware corporation

with and into

Advanced Magnetics, Inc.,
a Delaware corporation

It is hereby certified that:

1. Advanced Magnetics, Inc. ("*Parent*" or the "*Corporation*") is a business corporation organized and existing under the laws of the State of Delaware.

2. Parent owns all of the issued and outstanding shares of capital stock of AMAG Name Change Sub, Inc. ("*Subsidiary*"), which is a business corporation organized and existing under the laws of the State of Delaware.

3. Parent hereby merges Subsidiary with and into Parent.

4. In connection with the merger of Subsidiary into Parent, Parent hereby changes its name to AMAG Pharmaceuticals, Inc.

5. The following is a copy of the relevant recitals and resolutions adopted as of June 29, 2007 by the unanimous written consent of the Board of Directors of Parent under Section 141(f) of the Delaware General Corporations Law ("*DGCL*") approving the merger of Subsidiary with and into Parent under Section 253 of the DGCL:

WHEREAS: It is in the best interest for the Corporation to change its name to AMAG Pharmaceuticals, Inc.;

WHEREAS: the Corporation may change its name without stockholder approval under Section 253 of the DGCL by forming a subsidiary, causing that subsidiary to merge into the Corporation, and including in the certificate of ownership and merger a provision that the Corporation is changing its name;

WHEREAS: the Corporation desires to form a wholly-owned subsidiary, AMAG Name Change Sub, Inc., a Delaware corporation (the "*Subsidiary*"), to merge with and into the Parent, so that Parent will be the surviving corporation and can change its name pursuant to Section 253 of the DGCL;

WHEREAS: there has been submitted to and considered by the members of the Board an agreement and plan of merger (the "*Merger Agreement*") by and between the Subsidiary and Parent providing for the short-form merger (the "*Merger*") of the Subsidiary with and into the Parent pursuant to the DGCL and further providing that all of the assets and liabilities of the Subsidiary will become assets and liabilities of the Parent pursuant to DGCL Section 259 and that the

Parent will change its name to AMAG Pharmaceuticals, Inc. pursuant to DGCL Section 253(b); and

WHEREAS: the undersigned deems it advisable and in the best interests of the Corporation to approve and to consummate the Merger and that a Certificate of Ownership and Merger (the "*Merger Certificate*") be executed in accordance with DGCL Section 103 and filed with the Secretary of State of the State of Delaware and that any other appropriate documents and acts be executed, delivered and performed;

NOW, THEREFORE, BE IT:

RESOLVED: that Parent cause Subsidiary to be formed and issue 1,000 shares of its capital stock to Parent at its par value per share of $0.001 in exchange for $1.00 cash so that the Subsidiary will be a wholly-owned subsidiary of Parent;

RESOLVED: that Parent, a Delaware corporation and owner of all of the outstanding shares of Subsidiary, which is also a Delaware corporation, become a party to the Merger Agreement and undertake the Merger and thereby merge Subsidiary into the Corporation pursuant to the provisions of the DGCL and take ownership of all of the assets and assume all of the liabilities of Subsidiary;

RESOLVED: that Subsidiary shall be merged with and into Parent upon the effective date of the Merger pursuant to the DGCL and Parent shall continue its existence as the surviving corporation pursuant to the DGCL;

RESOLVED: that in connection with the Merger, Parent's name shall be changed from Advanced Magnetics, Inc. to AMAG Pharmaceuticals, Inc. and that Article I of Parent's Certificate of Incorporation, as amended, be further amended as permitted by Section 253 of the DGCL to reflect such name change;

RESOLVED: that the issued and outstanding shares of Subsidiary's capital stock shall not be converted in any manner, nor shall any cash or other consideration be paid or delivered therefor, inasmuch as Parent is the owner of all outstanding shares of Subsidiary, but each said share which is issued as of the effective date of the Merger shall be surrendered and extinguished;

RESOLVED: that officers of Parent are hereby authorized to enter into the Merger Agreement on behalf of Parent and to execute the Merger Certificate and cause it to be filed with the Delaware Secretary of State; and

RESOLVED: that the Board of Directors and the proper officers of the Corporation are hereby authorized, empowered and directed to do any and all acts and things, and to make, execute, deliver, file, and/or record any and all instruments, papers and documents which shall be or become necessary, proper or convenient to carry out or put into effect any of the provisions of the Merger herein provided for;

IN WITNESS WHEREOF, Advanced Magnetics, Inc. has caused this Certificate of Ownership and Merger to be signed by an authorized officer on the 24th day of July, 2007.

Advanced Magnetics, Inc.
a Delaware corporation

By: /s/ Joseph L. Farmer
Joseph L. Farmer
Its: General Counsel & Vice President of Legal Affairs

COMMONWEALTH OF MASSACHUSETTS)

) ss.

COUNTY OF SUFFOLK)

I hereby certify that the attached document is a true certified copy of the original document.

Anna M. Daley
Notary Public
My Commission Expires: August 30, 2013

(seal)

Attachment I

UNITED STATES PATENT AND TRADEMARK OFFICE

Facsimile Transmission

To:	Name:	BARBARA J. CARTER
	Company:	125 SUMMER STREET
	Fax Number:	16174430004
	Voice Phone:	
From:	Name:	ASSIGNMENT SERVICES BRANCH
	Voice Phone:	571-272-3350

37 C.F.R. 1.6 sets forth the types of correspondence that can be communicated to the Patent and Trademark Office via facsimile transmissions. Applicants are advised to use the certificate of facsimile transmission procedures when submitting a reply to a non-final or final Office action by facsimile (37 CFR 1.8(a)).

Fax Notes:

Pg#	Description
1	Cover Page
2	691.TXT
5	Document 1, Batch 1160390

USPTO ASSIGNMENT SYSTEM PROCESSING

Date and time of transmission: Thursday, January 31, 2008 8:19:56 PM
Number of pages including this cover sheet: 06

TO:BARBARA J. CARTER COMPANY:125 SUMMER STREET

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

500451509A

JANUARY 30, 2008

PTAS

BARBARA J. CARTER
125 SUMMER STREET
BROMBERG & SUNSTEIN LLP
BOSTON, MA 02110

UNITED STATES PATENT AND TRADEMARK OFFICE
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RECORDATION DATE: 01/29/2008 REEL/FRAME: 020431/0232
NUMBER OF PAGES: 7

BRIEF: CHANGE OF NAME (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: 1275

ASSIGNOR:
ADVANCED MAGNETICS, INC. DOC DATE: 07/24/2007

ASSIGNEE:
AMAG PHARMACEUTICALS, INC.
125 CAMBRIDGE PARK DRIVE, 6TH FLOOR
CAMBRIDGE, MASSACHUSETTS 02140

SERIAL NUMBER: 10386394 FILING DATE: 03/11/2003
PATENT NUMBER: ISSUE DATE:
TITLE: HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND USES THEREOF

020431/0232 PAGE 2

SERIAL NUMBER: 10410527 FILING DATE: 04/09/2003
PATENT NUMBER: ISSUE DATE:
TITLE: POLYOL AND POLYETHER IRON OXIDE COMPLEXES AS PHARMACOLOGICAL AND/OR MRI CONTRAST AGENTS

SERIAL NUMBER: 07650957 FILING DATE: 02/05/1991
PATENT NUMBER: 5160726 ISSUE DATE: 11/03/1992
TITLE: FILTER STERILIZATION FOR PRODUCTION OF COLLOIDAL, SUPERPARAMAGNETIC MR CONTRAST AGENTS

SERIAL NUMBER: 07694636 FILING DATE: 05/02/1991
PATENT NUMBER: 5262176 ISSUE DATE: 11/16/1993
TITLE: SYNTHESIS OF POLYSACCHARIDE COVERED SUPERPARAMAGNETIC OXIDE COLLOIDS

SERIAL NUMBER: 08043611 FILING DATE: 04/05/1993
PATENT NUMBER: 5490991 ISSUE DATE: 02/13/1996
TITLE: DIRECTED DELIVERY OF RADIOPROTECTANTS USING A RECEPTOR SPECIFIC CARRIER

SERIAL NUMBER: 07900686 FILING DATE: 06/17/1992
PATENT NUMBER: 5478576 ISSUE DATE: 12/26/1995
TITLE: ARABINOGALACTAN DERIVATIVES AND USES THEREOF

SERIAL NUMBER: 07936873 FILING DATE: 08/27/1992
PATENT NUMBER: 5336506 ISSUE DATE: 08/09/1994
TITLE: TARGETING OF THERAPEUTIC AGENTS USING POLYSACCHARIDES

SERIAL NUMBER: 08346142 FILING DATE: 11/29/1994
PATENT NUMBER: 5589591 ISSUE DATE: 12/31/1996
TITLE: ENDOTOXIN-FREE POLYSACCHARIDES

SERIAL NUMBER: 08260551 FILING DATE: 06/16/1994
PATENT NUMBER: 5554386 ISSUE DATE: 09/10/1996
TITLE: DELIVERY OF THERAPEUTIC AGENTS TO RECEPTORS USING POLYSACCHARIDES

SERIAL NUMBER: 08766597 FILING DATE: 12/12/1996
PATENT NUMBER: 5981507 ISSUE DATE: 11/09/1999
TITLE: POLYMERIC CARRIERS LINKED TO NUCLEOTIDE ANALOGUES VIA A PHOSPHORAMIDE BOND

SERIAL NUMBER: 09521264 FILING DATE: 03/08/2000
PATENT NUMBER: 6599498 ISSUE DATE: 07/29/2003
TITLE: HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES

SERIAL NUMBER: 07233177 FILING DATE: 08/16/1988
PATENT NUMBER: 5055288 ISSUE DATE: 10/08/1991
TITLE: VASCULAR MAGNETIC IMAGING METHOD AND AGENT COMPRISING BIODEGRADEABLE SUPERPARAMAGNETIC METAL OXIDES

SERIAL NUMBER: 07244432 FILING DATE: 09/14/1988
PATENT NUMBER: 4951675 ISSUE DATE: 08/28/1990
TITLE: BIODEGRADUBLE SUPERPARAMAGNETIC METAL OXIDES AS CONTRAST AGENTS FOR MR IMAGING

TO:BARBARA J. CARTER COMPANY:125 SUMMER STREET

020431/0232 PAGE 3

SERIAL NUMBER: 07409384 FILING DATE: 09/19/1989
PATENT NUMBER: 5069216 ISSUE DATE: 12/03/1991
TITLE: SILANIZED BIODEGRADABLE SUPERPARAMAGNETIC METAL OXIDES AS CONTRAST AGENTS FOR IMAGING THE GASTROINESTINAL TRACT

SERIAL NUMBER: 07475618 FILING DATE: 02/06/1990
PATENT NUMBER: 5102652 ISSUE DATE: 04/07/1992
TITLE: LOW MOLECULAR WEIGHT CARBOHYDRATES AS ADDITIVES TO STABILIZE METAL OXIDE COMPOSITIONS

SERIAL NUMBER: 07860388 FILING DATE: 03/30/1992
PATENT NUMBER: 5248492 ISSUE DATE: 09/28/1993
TITLE: LOW MOLECULAR WEIGHT CARBOHYDRATES AS ADDITIVES TO STABILIZE METAL OXIDE COMPOSITIONS

SERIAL NUMBER: 07863360 FILING DATE: 03/31/1992
PATENT NUMBER: 5219554 ISSUE DATE: 06/15/1993
TITLE: HYDRATED BIODEGRADABLE SUPERPARAMAGNETIC METAL OXIDES

SERIAL NUMBER: 07917567 FILING DATE: 07/20/1992
PATENT NUMBER: 5352432 ISSUE DATE: 10/04/1994
TITLE: ASIALOGLYCOPROTEIN RECEPTOR SPECIFIC COMPOSTIONS AND THEIR USE AS MRI CONTRAST AGENTS

SERIAL NUMBER: 07924121 FILING DATE: 08/03/1992
PATENT NUMBER: 5342607 ISSUE DATE: 08/30/1994
TITLE: RECEPTOR MEDIATED ENDOCYTOSIS TYPE MAGNETIC RESONANCE IMAGING CONTRAST AGENTS

SERIAL NUMBER: 07995111 FILING DATE: 12/22/1992
PATENT NUMBER: 5314679 ISSUE DATE: 05/24/1994
TITLE: VASCULAR MAGNETIC RESONANCE IMAGING METHOD COMPRISING NANOPARTICLES

SERIAL NUMBER: 08444136 FILING DATE: 05/18/1995
PATENT NUMBER: 5679323 ISSUE DATE: 10/21/1997
TITLE: HEPATOCYTE-SPECIFIC RECEPTOR-MEDIATED ENDOCYTOSIS-TYPE COMPOSITIONS CONTRAST AGENTS

ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

01/29/2008
500451509

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	CHANGE OF NAME

CONVEYING PARTY DATA

Name	Execution Date
Advanced Magnetics, Inc.	07/24/2007

RECEIVING PARTY DATA

Name:	AMAG Pharmaceuticals, Inc.
Street Address:	125 Cambridge Park Drive, 6th floor
City:	Cambridge
State/Country:	MASSACHUSETTS
Postal Code:	02140

PROPERTY NUMBERS Total: 21

Property Type	Number
Patent Number:	5160726
Patent Number:	5262176
Patent Number:	5490991
Patent Number:	5478576
Patent Number:	5336506
Patent Number:	5589591
Patent Number:	5554386
Patent Number:	5981507
Patent Number:	6599498
Application Number:	10386394
Application Number:	10410527
Patent Number:	5055288
Patent Number:	4951675
Patent Number:	5069216
Patent Number:	5102652

CH $340.00 5160726

TO:BARBARA J. CARTER COMPANY:125 SUMMER STREET

Patent Number:	5248492
Patent Number:	5219554
Patent Number:	5352432
Patent Number:	5342607
Patent Number:	5314679
Patent Number:	5679323

CORRESPONDENCE DATA

Fax Number: (617)443-0004
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 617-443-9292
Email: DDEramo@Bromsun.com
Correspondent Name: Barbara J. Carter
Address Line 1: 125 Summer Street
Address Line 2: Bromberg & Sunstein LLP
Address Line 4: Boston, MASSACHUSETTS 02110

ATTORNEY DOCKET NUMBER:	1275
NAME OF SUBMITTER:	Barbara J. Carter

Total Attachments: 5
source=DD1275001ChangeofName#page1.tif
source=DD1275001ChangeofName#page2.tif
source=DD1275001ChangeofName#page3.tif
source=DD1275001ChangeofName#page4.tif
source=DD1275001ChangeofName#page5.tif

Attachment J

DOCKETED

Electronic Acknowledgement Receipt	
EFS ID:	4157507
Application Number:	09521264
International Application Number:	
Confirmation Number:	1949
Title of Invention:	HEAT STABLE COLLOIDAL IRON OXIDES COATED WITH REDUCED CARBOHYDRATES AND CARBOHYDRATE DERIVATIVES
First Named Inventor/Applicant Name:	Ernest V. Groman
Customer Number:	02101
Filer:	Barbara J. Carter
Filer Authorized By:	
Attorney Docket Number:	1275/190
Receipt Date:	27-OCT-2008
Filing Date:	08-MAR-2000
Time Stamp:	20:48:09
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	DD1275190POA.pdf	130950 b9b464aa47fb7ac129aaaf0bbdf87947b7c3ceb8	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	130950

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

DOCKETED

Atty Docket: 1275/190 and 1275/700

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT NO:
6,599,498

ISSUE DATE:
July 29, 2003

Commissioner for Patents
Washington, D.C. 20231

POWER OF ATTORNEY BY ASSIGNEE

As an authorized representative of Assignee for the above-referenced patent, I hereby appoint each and all of the following attorneys to transact all business in the Patent and Trademark Office connected therewith:

SUNSTEIN, Bruce D.	27,234	CONWAY, John L.	48,241
ASHER, Robert M.	30,445	NOLL, Kathryn	48,811
MURPHY, Timothy M.	33,198	TUYTSCHAEVERS, Thomas	42,190
SAUNDERS, Steven G.	36,265	CARTER, Barbara J.	52,703
PETUCHOWSKI, Samuel J.	37,910	HESS, Robert	57,411
KLAYMAN, Jeffrey T.	39,250	HEYWARD, Moses	61,140
STICKEVERS, John J.	39,387	LOVELY, Jonathan	60,821
SANDVOS, Jay	43,900	MICHNA, Jacob	61,033
SMOLENSKI, Alex J.	47,953	ZWICK, David	41,393
BUCHANAN, Karen	37,790	BLAU, David	60,625
JAKOBSCHE, George	39,236		

The undersigned representative of Assignee hereby appoints the practitioner(s) associated with the Customer Number provided below to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
Barbara J. Carter	Barbara J. Carter
Bromberg & Sunstein LLP	(617) 443-9292
125 Summer Street	
Boston, MA 02110-1618	
Cust. No. 02101	

****

ASSIGNEE:

AMAG Pharmaceuticals, Inc.
100 Hayden Ave.
Lexington, MA 02421

By: Joseph L. Farmer Date: October 15, 2008

Name: Joseph L. Farmer

Title: General Counsel + SVP Legal Affairs

PTO/SB/96 (09-08)
Approved for use through 10/31/2008. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Groman, Ernest V. et al.

Application No./Patent No.: 6,599,498 Filed/Issue Date: 29 July 2003

Entitled: Heat Stable Colloidal Iron Oxides Coated with Reduced Carbohydrates and Carbohydrate Derivatives

AMAG Pharmaceuticals, Inc. , a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. X the assignee of the entire right, title, and interest; or
2. an assignee of less than the entire right, title and interest (The extent (by percentage) of its ownership interest is %)

in the patent application/patent identified above by virtue of either:

A. An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel , Frame , or for which a copy thereof is attached.

OR

B. X A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Groman, Ernest V. et al. To: Advanced Magnetics, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 010862 , Frame 0276 , or for which a copy thereof is attached.
2. From: Advanced Magnetics, Inc. To: AMAG Pharmaceuticals, Inc.
   The document was recorded in the United States Patent and Trademark Office at
   Reel 020431 , Frame 0232 , or for which a copy thereof is attached.
3. From: To:
   The document was recorded in the United States Patent and Trademark Office at
   Reel , Frame , or for which a copy thereof is attached.

Additional documents in the chain of title are listed on a supplemental sheet.

X As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Barbara J. Carter, #52,703/ October 27, 2008
Signature Date

Barbara J. Carter (617) 443-9292
Printed or Typed Name Telephone Number

Attorney for Assignee
Title